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RELATIONSHIPS OF SELECTED ENZYME SYSTEMS TO LIPIDS IN LIVERS OF YOUNG RATS FED SOY PROTEIN

by

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LIST OF ABBREVIATIONS

GPT	Glutamic pyruvic transaminase
SDH	Succinic dehydrogenase
RNAse	Ribonuclease
RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid
FAO-8	Fatty acid oxidase (substrate-caprylic acid)
FAO-16	Fatty acid oxidase (substrate-palmityl carnitine)
S 3.75	3.75% soy protein, without methionine
S 7.5	7.5% soy protein, without methionine
S 15	15.0% soy protein, without methionine
S 22.5	22.5% soy protein, without methionine
S 30	30% soy protein, without methionine
SM 3.75	3.75% soy protein, with methionine
SM 7.5	7.5% soy protein, with methionine
SM 15.0	15.0% soy protein, with methionine
SM 22.5	22.5% soy protein, with methionine
SM 30	30% soy protein, with methionine
S4M 7.5	7.5% soy protein, with 0.4% methionine
S4M 15	15.0% soy protein, with 0.8% methionine
L 7.5	7.5% lactalbumin, without methionine
L 15	7.5% lactalbumin, without methionine
LM 7.5	7.5% lactalbumin with methionine
LM 15	15% lactalbumin with methionine
PF	Protein-free

INTRODUCTION

Accumulation of hepatic lipids has been observed with variations in the quantity of dietary protein or modifications of the concentration of a single amino acid. The mechanism responsible for hepatic lipid infiltration with alterations in protein nutriture has not been elucidated. Investigations have been directed towards assessment of the regulatory role played by a number of processes in hepatic lipid metabolism. Rate of lipid synthesis, rate of oxidation or release of lipids from this organ has been implicated as contributing factors.

In this laboratory, the possibility of a relationship between accumulation of hepatic lipids and a decrease in fatty acid oxidase (FAO) activity has been explored. Crenshaw (1962) studied relationships among 1) quality of protein, 2) level of hepatic fat, and 3) activity of FAO in rats fed low levels of supplemented or unsupplemented wheat gluten or casein. Generally, mean hepatic FAO activities were greater in animals maintained on proteins supplemented with essential amino acids than those receiving isonitrogenous unsupplemented diets. However, the concentration of hepatic lipid was not significantly different among experimental groups, which may account for the fact that meaningful relationships between the levels of FAO and concentration of hepatic fat could not be established. These objectives were further pursued by Davidson (1963) with diets known to produce a considerable degree of hepatic lipid infiltration. Using diets of 15% protein from soy or lactalbumin, an inverse relationship between activity of FAO and concentration of hepatic fat was demonstrated.

Although lipid values were relatively greater in animals receiving unsupplemented soy diets, the degree of lipid infiltration was significantly reduced when 0.2 or 0.8% methionine was incorporated into 15% soy protein diets (Davidson, 1963). Apparently, a supplementary level as low as 0.2% had improved the amino acid mixture sufficiently to prevent excessive lipid accumulation.

On the basis of these studies, experiments were designed to test how varying levels of methionine in diets containing less as well as more protein than used before would affect hepatic lipids and FAO activity. In addition, attempts were made to correlate a number of enzymes related to hepatic metabolism with the nutritive value of soy protein diets varying in amounts of protein and methionine. The response of enzyme systems to variations in dietary protein has been useful in assessment of alterations in protein metabolism (Miller, 1950). The pattern of selected enzyme systems of animals maintained on graded quantities of casein has been categorized as: 1) increasing linearly, 2) increasing curvilinearly, 3) not changing, and 4) decreasing (Muramatsu and Ashida, 1962). The three enzyme systems reported to vary with variations of protein nutriture, succinic dehydrogenase (SDH), glutamic pyruvic transaminase (GPT), and ribonuclease (RNAse) were selected for study in animals fed a wide range of concentrations of soy protein with varying degrees of methionine supplementation.

REVIEW OF LITERATURE

The processes by which an animal organism maintains homeostasis are complex and interrelated. Several mechanisms usually function to control the concentrations of nutrients in organs and tissues. Thus, the quantity of hepatic lipids is dependent on numerous factors which function in the metabolism of fat. Deficiencies in certain nutritional factors, such as protein or choline, or consumption of diets containing an unsuitable amino acid pattern, may induce certain metabolic derangements with concomitant accumulations of neutral lipids in livers. Such livers have been referred to as 'fatty livers'. Generally, these large deposits of hepatic lipids are not considered to be a disorder by themselves because the condition is readily reversed in the presence of lipotropic factors. The term 'fatty livers' has been used loosely in the literature and may refer to lipid concentration barely above control values as well as to excessive lipid infiltration accounting for as much as 40% of wet liver weight.

Accumulation of hepatic lipids

Events which lead to infiltration of fat in livers have been extensively investigated. Pancreatectomized dogs treated with insulin and with a diet of lean meat and sugar developed pathological symptoms and death ensued. Postmortem studies revealed that the only organ seriously affected was the liver with depots of fat (Bliss, 1922). Reasoning that the observed changes were due to lack of exocrine secretions of the pancreas, Allan and associates (1924) were able to prevent the morbid symptoms by feeding raw beef pancreas. Other investigators attributed the changes to

a failure of hepatic function, particularly to the metabolism of fat. Lecithin or suet was incorporated into diets, but only lecithin alleviated the characteristic signs seen in insulin treated pancreatectomized dogs (Hershey and Soskin, 1931).

The effectiveness of lecithin as a lipotropic agent in rats was shown by Best, Hershey and Huntsman (1932). By including isolated fractions of lecithin in diets, choline was found to be the active lipotropic component (Best and Huntsman, 1932). Subsequent experiments demonstrated that a number of nutritional variations, such as increased fat intake, choline deficiency, or restriction of food intake, could lead to fatty infiltration; in many cases, the effect could be reversed by provision of dietary choline (Best and Huntsman, 1935).

Since these early studies, a number of nutrients, such as proteins, lipids or carbohydrates, have been demonstrated to play a role in the control of hepatic lipid accumulation. Studies on the role of types of fats have not yielded any conclusive results. The effectiveness of fats in controlling liver fat in weanling rats was related to chain lengths of their component fatty acids (Benton, et al., 1956). However, the relative quantity of saturated fatty acids also influenced the degree of hepatic lipid infiltration in young rats on low protein diets (Morris, et al., 1965). The type of carbohydrate incorporated in 9% casein diets affected the level of liver fat accumulated, but differences associated with variations in carbohydrates were eliminated by increasing the dietary casein to 11% (Harper, et al., 1953). Diets of low protein content (Best and Huntsman, 1935) or composed of unsuitable amino acid patterns (Harper, et al., 1954)

are known to induce lipid accumulation. These factors will be discussed in a separate section.

Non-dietary factors, such as sex, age, or toxic agents, also modify the mechanism which controls lipid infiltration. Force-feeding of choline-containing diets devoid of methionine induced fatty livers in adult female, but not in adult male rats, whereas (Sidransky and Farber, 1958b) force-feeding a diet devoid of threonine induced fatty livers in both sexes (Sidransky and Farber, 1958a). Griffith and Wade (1939) reported that rats weighing 124 g developed only slightly fatty livers on diets which produced markedly high degrees of lipid infiltration in rats weighing 40 g. The numerous toxic agents which will provoke fat infiltration in livers will not be reviewed in this discussion.

Thus, alterations of one of several processes which are operative in metabolism could result in the accumulation of hepatic lipids. The susceptibility of animals to development of fatty livers induced by dietary changes is modified by sex and rate of growth. Although a complex interaction of many factors may contribute to the control of lipid concentration, only those factors involving protein and methionine alterations will be discussed.

Dietary amino acids and hepatic lipid accumulation

That proteins might have lipotropic potentialities was suggested by the observation that an anticipated rise in hepatic lipids in rats on a choline deficient diet could be prevented by including 20% casein in the diet (Best and Huntsman, 1935). Subsequently, effects of varying dietary

protein from 0 to 50% on development of fatty livers were investigated. With low protein diets, an inverse relationship was found between the percent of dietary protein and concentration of hepatic lipids. However, with dietary protein in excess of 20%, a diminishing rate of lipid infiltration was apparent. No relation was observed between amount of lipid in livers and that in body depots (Channon and Wilkinson, 1935).

The effectiveness of different proteins as lipotropic agents varies. The isonitrogenous substitution of gelatin for a portion of casein produced increased accumulation of hepatic lipids (Best, et al., 1936). Lipid concentrations in rats on 18% casein diets were lowered by inclusion of choline, but partial substitution of casein by other proteins did not lower hepatic lipids whether a choline supplement was given or not. The experimenters found that the dietary casein content had to exceed 40% before lipid values were significantly lowered. At this high level of casein, excessive accumulation of hepatic lipid was apparent when gelatin was used to contribute part of the nitrogen, but was not evident with substitution of casein by lactalbumin or fibrin. If methionine or choline was given with gelatin, hepatic lipids were somewhat depressed, but the level was still higher than that seen in controls (Griffith, 1941). Further, purified diets of fibrin, casein, and/or albumin as protein sources at concentrations of 5, 10, or 15% did not prevent fatty infiltration of livers of young rats unless choline was included (Griffith and Wade, 1939). Thus, animals on low protein diets were especially vulnerable to excessive deposits of hepatic lipids.

Because animals on high protein diets were protected from hepatic fat infiltration to a greater degree than those on low protein diets, the lipotropic activity of individual amino acids was investigated. None of the amino acids studied by Beeston and Channon (1936), had lipotropic activity in mature rats. However, cystine caused the level of hepatic lipids to rise. The 'antilipotropic' effect of cystine in weanling rats was confirmed by Mulford and Griffith (1942). These investigators demonstrated that cystine or methionine plus cystine stimulated growth in rats thus increasing the need for choline. There was no evidence that cystine exerted a toxic effect above the amounts needed to promote growth. Mulford and Griffith reported that in the absence of choline 30% casein with supplemental methionine was necessary to prevent signs of choline and cystine deficiency in young rats.

Studies in mature animals on low protein, high fat diets showed that the addition of methionine depressed the level of hepatic lipids (Tucker and Eckstein, 1937). Provision of methionine supplements equivalent to the amount found in 20% casein prevented fatty livers in rats fed 15 times the amount of cystine present in 20% casein diets. It was also found that methionine, provided as the free amino acid, was more effective in prevention of liver fat accumulation than an equivalent amount given in the form of intact casein (Treadwell, et al., 1942). Thus, the lipotropic activity of methionine was established.

A relationship between choline and methionine was suggested by observations that young rats on diets adequate in choline could utilize

homocystine in place of methionine (duVigneaud, et al., 1939). It was postulated that rats were able to transmethylate the group supplied by choline onto the carbon skeleton provided by homocystine. Subsequently, rats fed a choline-deficient diet containing methionine with a deuterium-labelled methyl group incorporated the label into its tissue choline. Apparently, methionine could contribute its methyl group for choline synthesis. This illustrated the existence of a reversible pathway between the synthesis of methionine and choline (duVigneaud, et al., 1941).

Treadwell (1948) suggested that the effect of choline may be to spare methionine. When a supplement of 0.5% methionine was given to weanling rats on an 18% protein and low choline diet, depression of growth rate, hemorrhagic kidneys, and hepatic lipid accumulation were observed. Only the latter symptom was visible when methionine supplement was increased to 1%. Under conditions of this experiment, it was suggested that at least 1.3% methionine was needed to prevent lipid accumulation in the absence of choline.

The recognition of protein, and subsequently of methionine, as important factors in regulating deposition of hepatic lipids led to the question of whether lipotropic activity of a protein was solely determined by its content of sulfur-containing amino acids. Early investigations did not settle the issue, primarily because of variations in experimental conditions. In order to investigate the problem, concentrations of hepatic lipids in three groups of animals were compared (Beveridge, et al., 1945). Group 1 received graded amounts of casein from 10 to 35%. The second group was fed methionine and cystine in amounts proportional to that found in

casein diets of group 1. The third group was maintained on all essential amino acids (including methionine and cystine) in amounts comparable to that found in diets of group 1. All diets were then supplemented with gelatin so that total protein content was 35%.

Animals receiving purified essential amino acids had higher lipid concentrations than groups maintained on either casein or sulfur-containing amino acids. Comparison of animals fed sulfur-containing amino acid diets with those maintained on casein showed that methionine and cystine were more effective in the prevention of lipid accumulation than casein at levels below 22% protein. This investigation suggested that sulfur-free essential amino acids influenced growth and maintenance and thus modified the methionine left for regulation of deposition of hepatic lipids. When the amino acid pattern was sufficient to promote growth, the amino acids were directed toward growth. If, however, the amino acid pattern of the diet did not promote growth, the available sulfur-containing amino acids were directed toward regulation of lipid content of the liver. Animals were able to grow on low levels of casein leaving fewer amino acids for regulation of lipid. With high levels of dietary casein, the excess methionine was used for regulation of lipid deposition.

Liver lipid accumulation has also been observed in animals fed diets deficient in a single amino acid such as tryptophan (Spector and Adamstone, 1950) or phenylalanine (Samuels, et al., 1951). Singal and associates (1953) studied the role of threonine as a lipotropic agent. Weanling rats on adequate choline diets with 20% casein had normal hepatic lipid concentrations but those on a 9% casein diet with choline showed large lipid

deposits. Although choline or methionine had some lipotropic action, these compounds were not as effective in preventing lipid accumulation as a supplement of threonine. However, the removal of choline destroyed the effectiveness of threonine. Similar results were obtained by feeding a mixture of purified amino acids simulating 9% casein. These investigators suggested the existence of two separate, yet related, phenomena in the production of fatty livers. One was deficiency of methyl groups in which the provision of threonine was ineffective and the second was a threonine deficiency in which choline at very high levels was effective.

Harper and coworkers have published a series of studies on fatty livers induced by feeding low protein diets which threw more light on the role of threonine in lipid deposition. Accumulation of hepatic lipids was not seen when a 9% casein diet was fed, but was apparent when a methionine supplement was included (Harper, et al., 1953). The concentration of hepatic lipid induced by the methionine supplemented casein diet was depressed by adding 2% casein, 6% gelatin, threonine or glycine. The addition of other selected amino acids did not lower hepatic lipid levels. The apparent 'antilipotropic' effect of methionine was presumably due to a precipitation of a deficiency of the second limiting amino acid of casein, threonine, when the sulfur-containing amino acid was incorporated into a diet with low amounts of casein. However, the curative effect of threonine could only be demonstrated when the diet contained close to the stated requirement of choline or sufficient methionine to replace choline (Harper, et al., 1954).

Methionine deficiency has also been known to induce fatty livers

(Forbes and Vaughn, 1954). Lyman and associates (1964) presented evidence indicating that lipid accumulation in methionine-deficient animals was due to impaired removal of lipids from the liver. A mixture of purified amino acids simulating 18% casein without methionine produced fatty livers in female animals, weighing 160 g, but not in male rats, weighing 180 g. Acetate-1-^{14}C studies and measurement of dilution of triglycerides by linoleic acid demonstrated that fatty acid synthesis was not impaired in methionine deficiency. However, female methionine-deficient rats showed significantly lower serum cholesterol and phospholipid levels than controls, suggesting a defect in transport of lipids from liver to blood.

In summary, excessive lipid accumulation has been observed when dietary proteins provide an unsuitable assortment of amino acids. Producing an amino acid imbalance, especially when the diet is low in protein and marginal in choline, has also been shown to increase lipid deposition.

FAO and hepatic lipids

The importance of the liver in metabolism of lipids is evident from the roles which this organ assumes in synthesis, oxidation, and release to other tissues of fatty acids and related compounds. Excessive accumulation of hepatic lipids has been attributed to derangement of any one, or more, processes such as decreased transport of triglycerides from the liver (Olson, et al., 1958), reduced fatty acid oxidation (Artom, 1958), and increased fatty acid synthesis (Lyman, et al., 1964). Indeed, Stetten and Salcedo (1944) found different reasons for the existence of large deposits of hepatic fat in animals given diets of 10% casein. Rats fed choline-deficient diets exhibited impaired transport of fatty acids from

the liver; rats fed extra cystine showed an increase in fatty acid synthesis; and mice injected with hormones demonstrated an elevated mobilization of fat from body depots to the liver. However, only investigations exploring fatty acid oxidation will be reviewed since oxidation was the only process studied in the present experiment.

In this laboratory depression of hepatic fatty acid oxidase activity and accumulation of hepatic lipids have been associated with changes in protein nutriture due to inadequate supply of proteins, proteins unbalanced with respect to amino acid composition, or restriction in energy value of the diet. Weanling male rats receiving 9% casein showed FAO activity equivalent to 75% of that in rats maintained on control diets, whereas lipid values associated with experimental diets were greater than those of controls (Crenshaw, 1962). A significant correlation between high hepatic lipid values and low fatty acid oxidase activity was reported with diets containing 15% lactalbumin and/or soy protein (Davidson, 1963). Hepatic FAO in adult rats was sensitive to food restriction and protein depletion (Thorp, 1966).

The rate of oxidation of hepatic fatty acid was reduced in choline deficiency. When livers from rats maintained on low choline-low protein diets were incubated with labelled long chain fatty acids, CO_2 production was reduced. This suggested that the lipotropic effect of choline was largely due to enhancement of oxidation (Artom, 1953). However, <u>in vitro</u> addition of choline or related compounds did not stimulate fatty acid oxidation, suggesting that the effect of choline was not at the molecular level.

The work of Dianzani and Morinari (1961) supported the theory that malfunction of hepatic oxidative process may be responsible for the fat accumulation characteristic of choline deficiency. Low rates of mitochondrial oxidation of octanoic acid were seen in adult rats receiving injections of hepatoxic agents or choline-deficient diets. The administration of choline lowered the level of hepatic lipids and increased the octanoic acid oxidation. The depressed oxidation associated with choline-deficient diets was attributed to either a decrease in concentration of mitochondrial matter or lack of necessary co-factors.

Altered concentrations of certain co-factors required for the oxidation of fatty acids have been observed in fatty livers induced by carbon tetrachloride, white phosphorous, or choline deficiency. Reduction in total pyridine nucleotides, in the ratio of oxidized to reduced forms of pyridine nucleotides (PN/PNH) (Dianzani, 1955) as well as in ATP concentration (Dianzani, 1957), were noted when fatty livers were compared with control livers. A decrease in hepatic pyridine nucleotides was also observed in threonine-deficient animals (Arata, et al., 1964). Since both DPN and ATP are obligatory co-factors in oxidation of fatty acids, investigations were conducted to determine if alterations in oxidation of fatty acids, in metabolism of adenosine polyphosphates and pyridine nucleotides, and in concentration of hepatic lipids were interrelated. Injection of carbon tetrachloride brought elevation of liver fat prior to loss of DPN dependent oxidative function and ATPase transformation (Rechnagel and Anthony, 1959). This suggested that the primary biochemical lesion following carbon tetrachloride injection was not related to changes in pyridine nucleotide or

ATP concentration. Investigations with rats who had ingested carbon tetrachloride revealed a fall in concentration of plasma triglycerides and lipoproteins. The defect may be in the synthesis of hepatic lipoproteins or their release into the plasma. This would apply especially to the very low density lipoproteins (Lombardi and Ugazio, 1965). The lesions seen with carbon tetrachloride treatment may be caused by interference with synthesis of the protein moiety (Schotz, et al., 1964).

In threonine deficiency, however, decreases in fatty acid oxidase activity preceded increases in hepatic lipids (Arata, et al., 1964). In addition, after 2 weeks on a threonine-deficient diet, livers of rats showed depressed activities of the DPN-cytochrome c reductase system, as well as a decreased supply of ATP, and a lowering of the PN/PNH ratio. These changes indicated a reduction in the availability of co-factors for oxidation and an accumulation of materials favorable for fat synthesis in threonine deficiency.

The administration of the ethyl analog of methionine precipitated fatty livers in female, but not in male rats (Artom, 1959). Livers from treated and untreated groups were able to synthesize phospholipids, but homogenates of fatty livers oxidized stearate-1-^{14}C slowly. In another study depression of the palmitate-1-^{14}C oxidation rate was accompanied by a general decrease in metabolic processes. This was documented by a drop in rates of initial incorporation of ^{14}C into neutral fatty acids and of removal of fatty acids from the liver (Olivecrona, 1962).

Thus, not only was the oxidation of fatty acids depressed in ethionine-treated rats, but also apparent were depressions of initial incorporation

of the label into neutral fatty acids and of transport of fatty acids from the liver.

The effect of a high fat diet on fatty acid oxidation has also been studied. An increased fatty acid oxidase activity was seen in livers of animals maintained on a 40% fat diet. But in contrast to findings discussed above fatty infiltration in the liver did not occur (Rikans, et al., 1964). The elevation of enzyme activity was described as an adaptive response by the animals to adjust to a high lipid intake. When excessive amounts of niacin were incorporated into the high fat diet, the adaptive response was not apparent and there was a concomitant accumulation of hepatic lipids. Since the excretion of the end product of niacin metabolism requires methylation, a combination of high niacin and high fat could cause an increase in the animal's requirement for choline. However, Rikans and associates attributed the increase in lipid deposit to factors other than the lack of choline since, in contrast to choline deficiency, the fatty acid oxidase activity associated with high niacin intake was not altered and was comparable to that of controls.

Succinic dehydrogenase

Mitochondria contain a complement of enzymes necessary for the oxidation and storage of energy containing compounds. One of the mitochondrial systems whose component enzymes have been fairly well characterized is succinic oxidase. Evidence has been presented that in protein deficiency the rate limiting reaction of the succinic oxidase system is the initial dehydrogenase step (Williams, 1964). The level of activity of succinic dehydrogenase (SDH) could presumably be an index of energy metabolism.

Thus, the activity of this enzyme has been affected by variations in fat, carbohydrate, and other nutrient intakes. Only the influence of dietary variables closely related to protein on SDH will be discussed.

An extensive investigation of the response of SDH to varying protein intakes was conducted by Muramatsu and Ashida (1962). Although the protein content of the diets varied from 0 to 60%, growth and SDH activity curves of male weanling rats reached a plateau with a 25% casein diet. For 0 and 10% protein diets SDH activity was 10 and 50% of the maximum level.

Since the values for the two parameters did not differ with a 2 or 4 week experimental period, subsequent studies designed to study SDH response to graded levels of protein from a variety of sources, were limited to the shorter feeding period. Stepwise increments of wheat gluten and soy protein diets caused nearly linear increases in SDH activity, but maximum responses were not achieved even when the protein content of the diets was raised to 40% (Muramatsu and Ashida, 1963). A plateau in the SDH pattern of response was seen when high concentrations of the other two proteins tested were included in the diet. Maximum SDH concentration was obtained with diets containing 16% egg albumin and 24% fish meal. However, maximum activity induced by casein and egg albumin was less than that associated with soy protein or fish meal. Unlike the responses evoked by casein, the quantity of protein which produced maximum body weight did not coincide with that for maximum SDH activity. Thus, the mechanism by which the various proteins stimulate activity needs clarification.

Further analysis of the changes of SDH concentration as a function of alteration in quantity of soy protein revealed that elevating the protein

content from 9 to 35% brought a twofold enhancement in SDH activity. The SDH concentration associated with 35% soy protein diets was nine-tenths of the maximum.

The deprivation of dietary protein produced a drastic decrease in SDH activity (Williams, 1961). Protein depletion for a duration of 56 days was accompanied by a 60% decrease in activity in adult rats (Williams, 1964). A slight increase in SDH activity was observed after day 56 until realimentation with 20% casein was initiated on day 102. The precipitous decrease in SDH activity in protein-deficient rats was attributed presumably to the lack of protein rather than voluntarily decreased food consumption, since enzyme activity of pair-fed and ad lib controls were similar. The necessity of protein in maintaining the SDH system was further illustrated by the rapid rise in activity with repletion.

SDH activity may be a function of the presence of specific amino acids (Williams, 1961). The addition of methionine to a protein-free diet curbed the total drop in activity evident in protein depletion (Williams, 1964). The protective effect of methionine on SDH activity was evident after a prolonged experimental period when SDH concentration associated with diets including methionine did not differ from controls receiving 20% casein.

Since methionine apparently preserved the level of SDH activity, other amino acids were added individually to protein-free diets. Only methionine and cystine were found to protect SDH and the effects of the two amino acids appeared to be equivalent. The activity of SDH depends on a functional sulfhydryl group, and methionine and cystine could exert their effect by

protection of the sulfur-containing groups. However, the fact that methionine promoted the conservation of liver protein during protein depletion indicated that an exogenous source of these amino acids may serve more than protecting the sulfhydryl group.

SDH is an integral part of the succinic oxidase system and the activity of a component enzyme does not necessarily reflect the activity of the entire system (Williams, 1964). The response of SDH and succinic oxidase to protein depletion, repletion and the presence of sulfur-containing amino acids were found to be identical (Williams, 1964). These results suggest that the rate limiting step of succinic oxidase is presumably the initial dehydrogenation reaction. However, the degree to which SDH activity can be considered an indicator of energy metabolism is not known because one cannot assume that all SDH activity measured in vitro represent functional activity in vivo.

Young rats, sequentially fasted and repleted showed striking variability in SDH activity (Soberon and Sanchez, Q, 1961). The concentration of SDH decreased during the first quarter of a 4 day fast and increased above control values during the remaining days of food deprivation. Repletion with either 17% casein or zein returned the concentration to control levels. Total activities followed a similar pattern of response, although to a less marked extent. Thus, SDH concentration appeared to increase during fast and decrease during repletion.

Partial hepatectomy was performed on young rats to observe regeneration of liver of normal composition but of reduced size (Sanchez, Q, et al, 1961). SDH activity declined during the first 24 hours postoperatively

and rose slowly toward control level after that. When 17% casein or zein diets was given, animals fed the zein diet for 8 preoperative days appeared to have higher SDH activity during early post operative days. However, at the end of the 4th postoperative day SDH concentrations were not different among all three groups.

The response of the hepatic succinic dehydrogenase system to dietary variations has been investigated in this laboratory. Marked differences in concentrations of SDH activity were observed. Nirmala (1964) reported that a combination of rice and peanut stimulated greater SDH activity than was provided by the individual proteins. Increases in SDH activity were observed when lactalbumin was increased from 5 to 15% (Garcia and Roderuck, 1964), and when the variety of protein mentioned previously was raised from 4 to 8% (Nirmala, 1964). On the other hand, changes in proportions of fat or carbohydrate in the diet did not result in alterations of SDH activity. The enzyme remained refractory to the substitution of 20% fat for carbohydrate in a fat free diet (Thorp, 1966). However, regardless of the types of energy yielding nutrients included, restriction of total food intake was associated with increased activity while liver size decreased (Thorp, 1966; Garcia and Roderuck, 1964). Thus, these studies demonstrated that, in adult animals, hepatic SDH was sensitive to variations in quality and quantity of protein and was protected preferentially when food energy was restricted.

GPT

A characteristic feature of metabolism in animals is the maintenance of a stable chemical pattern of body tissues and fluids. The biochemical

transformations of proteins and their derivatives are therefore controlled and interrelated with metabolic processes of other energy yielding nutrients. The interplay of these processes is dependent on numerous factors, among which is the process of transamination (Braunstein, 1947).

The mechanism of transamination reactions requires pyridoxal phosphate as a coenzyme and amino acids and their corresponding keto acids as substrates. Activity, specificity, and number of transaminases vary from species to species (Snell and Jenkins, 1959).

Changes in hepatic glutamic pyruvic transaminase (GPT) in rats have been reported with alterations of several dietary variables. Protein depletion (Som and Esh, 1963; Awapara, 1953) or deficiencies of vitamins such as riboflavin (Mookerjea and Jamdar, 1962) or pyridoxine (Ning, et al., 1966) have produced changes in transamination. In addition, activity of this enzyme was increased when animals were subjected to varying protein intakes (Beaton, 1963) or fasting (Soberon and Sanchez, Q., 1961). In the present review, only influences closely related to dietary protein on the specific hepatic transaminase, glutamic alanine or glutamic pyruvic transminase, in rats will be discussed.

Progressive elevation of GPT activity accompanied increases in dietary protein intakes (Beaton, 1963; Muramatsu and Ashida, 1962). Young male rats fed 0, 18, 35, 50, or 75% casein diets showed changes in GPT activity which could be described as 1, 1.8, 2.4, 4.1, and 7.5-fold increases, assuming the activity of those fed 0 protein to be unity (Rosen, et al., 1959). Since the glucocorticosteroids have been known to induce glucogenic action and a negative nitrogen balance, studies were conducted to ascertain

whether the effect of protein was mediated through the adrenal glands or through metabolic adaptations of animals to protein per se. Progressive elevation of protein intakes produced parallel changes of GPT activity in adrenalectomized rats, but the maximum response was only two-thirds of the activity observed in intact rats. Administration of hydrocortisone to adrenalectomized rats restored activity of animals fed diets in excess of 25% protein to control levels. Thus, maximum GPT response appears to be a function of the additive effects of both dietary protein and hydrocortisone.

Other conditions of increased rates of gluconeogenesis, such as fasting, were associated with a rise in GPT activity (Rosen, et al., 1959). Conditions which stimulate gluconeogenesis may initially influence some aspect of protein synthesis. A depression of this process may cause an increase in the amino acid pool. The accumulation of amino acids may stimulate enzyme synthesis by substrate induction.

Schimke (1962) explored the interrelationship of several enzyme systems. In his study, GPT per g liver was found directly proportional to the daily consumption of protein by male weanling rats fed 15, 30, and 60% casein diets. Alterations in the response of GPT were comparable to variations in urea cycle enzymes under similar dietary conditions. Since there was a close correlation between urea cycle enzyme activity and dietary protein or excretion of urea, the level of GPT may possibly be related to the degree of protein catabolism.

The quality of protein may influence the degree of change in GPT in animals fed low levels of dietary protein. GPT activity paralleled increases in dietary protein from 6 to 12 to 18%, when protein was derived from casein or Bengal gram (Som and Esh, 1963). However, the concentration

of GPT was lower with 6 and 12% pulse protein than with isonitrogenous amounts of casein. When the protein levels were increased to 18%, enzyme activity associated with the two diets was not different. This suggested that at marginal intakes of protein GPT activity may also reflect the quality of protein.

The effects of partial food restriction and starvation on GPT activity have also been investigated. GPT activity was enhanced by 5 days of starvation, although partial food restriction had little effect (Waldorf, et al., 1963). No change in GPT activity was observed during the first 24 hours of fast. Possibly residual food in the gastrointestinal tract was being absorbed so that a supply of nutrients was available during the early stages of fasting. After 24 hours, GPT continued to rise for the 5 day experimental period. On the other hand, elevation of GPT followed an initial decline in activity during fasting (Soberon and Sanchez, Q., 1961). The rise peaked at 48 hours, and declined to control levels by the fourth day at which time realimentation was instituted. GPT per unit protein was unchanged by repletion with either zein or casein diets.

Although the degree of enhancement and the duration of elevation of GPT activity during a fast were varied, fasting did induce an increase in GPT activity. Since a fasting animal derives energy by catabolizing body tissue, an association between the elevation of activity and protein catabolism was suggested.

Because fasting presumably produces changes in the concentrations of liver components, such as protein, glycogen, fat, water, and electrolytes, a sub-total hepatectomy was performed to follow the course of repletion of

liver when its size was diminished (Sanchez, Q., et al., 1961). A decline in GPT activity during the first 24 postoperative hours and a rise to preoperative levels within 96 hours were seen. The initial decline may have been due to the stress of surgery, but support for this idea is missing since sham operations were not performed. The rise to control levels indicated a rapid recovery of the GPT system.

The sensitivity of the GPT system does not appear to be influenced by the age of the animal. Schimke (1962) reported similar activities in rats weighing 50 or 150 g fed isonitrogenous casein diets. GPT values in 5 and 14 month old rats fed 25% casein did not differ (Waldorf, et al., 1963). In contrast, when the dietary protein was increased to 80%, young animals had twice as much activity as older animals (Waldorf, et al., 1963).

Thus, GPT activity seem to increase with elevation of protein intake or protein catabolism.

RNA and RNAse

Investigations of the nutritional regulation of ribonucleic acid metabolism in rats have been limited primarily to studying the effects of feeding protein-free diets. Protein depletion has been associated with depressed levels of ribonucleic acid (Campbell and Kosterlitz, 1948; Munro and Clark, 1960; Girija, et al., 1965).

Rats given a protein-free ration showed a rapid decrease in hepatic RNA concentration and uptake of ^{32}P into RNA during the first day (Munro and Clark, 1960). However, the frank protein deficiency induced a compensatory increase in the production of RNA so that the absolute rate of synthesis was elevated. The depression of uptake could be explained by

assuming reutilization of nucleotides which resulted from the breakdown. Analysis of subcellular fractions demonstrated that part of the RNA became unstable with withdrawal of protein and that the RNA of the endoplasmic reticulum was the labile species.

The postulation that RNA catabolism was elevated in protein-depleted rats was supported by the findings of Girija and associates (1965). These investigators fractionated livers of control and protein depleted rats. Nuclear, transferal, and ribosomal RNA fractions were analyzed for ^{32}P uptake from labelled orthophosphoric acid. The nuclear RNA fraction took up the label more rapidly than did transferal and ribosomal fractions. The rapidly exchanging fraction was synthesized at an increased rate while the production of transferal and ribosomal RNA was depressed in protein-depleted rats. Moreover, these effects were reversed by incorporation of protein into the diets.

The necessity of having all the essential amino acids available to sustain nucleic acid formation was shown by a decreased ^{14}C-glycine incorporation into RNA with the omission of tryptophan (Munro and Clark, 1960). Evidently, when the supply of amino acids to the liver became nutritionally unsatisfactory, there was a reduction in polysome population. The differences in polysome population which occurred in response to the absence or presence of tryptophan were still apparent in rats treated with actinomycin D.

To investigate the mechanism responsible for an observed reduction of ^{14}C-leucine into acid soluble proteins in protein-depleted rats, the quantity and stability of polysomes were determined (Mandel, et al., 1966).

Although the amount of polysomes was less with the feeding of a protein free diet, the values could be restored to control levels by feeding an adequate diet. The synthetic ability of polysomes from control and protein-depleted rats was similar. Thus, the depression of protein production seen in frank protein deficiency was probably due to increased breakdown rather than decreased synthesis of RNA.

The studies by Allison and associates demonstrated that an increase in dietary protein produced increases in RNA content and decreases in RNAse activity. Results of hepatic RNAse analysis of rats fed 0, 12, and 30% casein illustrated that concentrations of RNAse were considerably higher in adult protein depleted rats than in those receiving protein (Zigman and Allison, 1959). These observations were verified in a study in which the range of dietary proteins was expanded from 0 to 50% casein. The RNAse activity tended to decrease as the amount of dietary protein was increased (Allison, et al., 1961). Protein depletion caused an increase in RNAse activity and a decrease in RNA content and RNAse inhibitor suggesting that the rise in RNAse activity in protein deprivation may be due to decreased inhibition of enzymic activity (Girija, et al., 1965).

The concentration of RNA and RNAse appears to be influenced by the type of protein fed. Weanling rats were maintained on 4 levels of protein from 4 sources: egg albumin, casein, cottonseed flour or wheat gluten (Allison, et al., 1962). The tendency for RNAse activity to decline as amounts of protein was increased was again evident. However, the activity induced by feeding different proteins was not similar. Low gluten diets stimulated higher activity than high gluten diets; but the latter values

were comparable to those produced by low quantities of other proteins. The activity of RNAse in rats fed cottonseed flour was altered very little by increasing the protein, but a more pronounced depression was apparent in those fed increasing casein or egg albumin (Allison, et al., 1962). These observations supported the suggestion that an interrelationship between RNA and RNAse played a role in controlling the rate of protein biosynthesis.

In contrast, other workers have failed to establish a relationship between dietary protein intake and RNAse activity. Young, mature animals fed 0 to 30% casein with or without methionine showed elevated concentrations of RNAse with high levels of protein, but no difference was noted when concentration was related to hepatic nitrogen (Matsuo, et al., 1966). In young rats, no difference in RNAse activity could be attributed to diets of 20 % casein, or free of protein. Moreover, fractionation of liver homogenate into mitochondria, microsomes, and supernatant revealed no specific effect of protein alterations on RNAse activity (Corcos Benedetti, et al., 1966a). Similar results were observed in older rats (Corcos Benedetti, et al., 1966b). Since the fractions had been treated with a surface active compound, these investigations suggested that protein deprivation increased the release of the enzymes from the mitochondria and microsomes.

In summary, the effect of protein nutriture on RNAse activity is equivocal since reports indicate that the concentration of this enzyme may increase, remain unchanged, or decrease as the protein content of the diet increases.

Influence of soy protein on growth

Interest in the use of soybean as a protein source stimulated research

related to this food in the early 1900's. Analyses revealed that the choline and calcium contents were low. Studies on nitrogen utilization and growth rates of weanling rats indicated that the nutritive value of soybeans could be improved by heating (Osborne and Mendel, 1917). Differences in rates of growth could probably not be attributed to palatability since weanling rats fed either raw or cooked soybeans grew equally well for a short time. Furthermore, the growth inhibition associated with raw soybeans was ameliorated by inclusion of dietary casein (Hayward, et al., 1936).

Sulfur and nitrogen balance studies by Johnson and associates (1939) confirmed the superior nutritive value of heated soybeans. These authors suggested that soy contained sulfur and nitrogen complexes which were more easily assimilated when heated. Previously, the importance of incorporating the sulfur-containing amino acids into soy diets was indicated by the work of Mitchell and Smuts (1932). These authors observed that cystine supplementation improved the growth of weanling rats fed diets of 10% protein from soy. However, the addition of methionine, or of methionine and cystine, but not cystine alone, reversed the weight loss seen when an amino acid mixture lacking these components was given. Thus, the essential sulfur-containing amino acid was shown to be methionine (Womack, et al., 1937).

Ham and Sandstedt (1944) reported that unheated soy contained a heat-labile compound which inhibited trypsin. However, the antitryptic factor may be responsible for only part of the growth retardation associated with raw soy meal. A second inhibitory factor in unheated soy (Liener, 1953),

later identified as a hemagglutinating substance (Liener, 1958), also inhibited growth. The growth depressing ability of both factors was apparently destroyed by autoclaving.

Although autoclaving soy proteins improved growth rates, the addition of sulfur-containing amino acids to heated soy meal stimulated further growth. That heat treatment increased the availability of cystine and perhaps of the entire protein was suggested by Hayward and Hafner (1941). In the same investigation, methionine was established as the most limiting amino acid of soy protein. This observation was confirmed by Barnes and coworkers (1962) who also reported that the methionine requirement could not be met by adding cystine to soy. Berry and associates (1962) listed the first three limiting amino acids of soy as methionine, lysine, and threonine, respectively.

The methionine requirements of growing rats fed soy diets have been investigated. With 12% protein diets, animals on heated meal required 0.15%, while those on unheated meal required 0.19% methionine (Borchers, 1961). These objectives were also pursued by Barnes and coworkers (1962) in animals fed diets containing 15 or 25% protein with ample cystine or increasing amounts of unsupplemented soy protein. After correcting for unabsorbed methionine, maximal growth was achieved when 0.17 to 0.19% available methionine was provided either as supplemental or food methionine. Weanling rats on a mixture of amino acids which supported 4.5 g gain per day required 0.16% methionine in the presence of excess cystine (Rama Rao, et al., 1961). Thus, under conditions where cystine is not limiting, methionine requirement for soy diets ranging from 12 to 25% protein has been

found to range from 0.15 to 0.19% available methionine.

A diet of 50% unheated soy flakes provides approximately 0.18% methionine. Incorporation of 0.3% methionine into diets containing less than 50% unheated soy flakes produced greater gains than an equivalent amount of unsupplemented protein (Barnes, et al., 1962). However, the addition of the amino acid to diets of 60 to 70% unheated soy flakes produced a depression of growth rates. Presumably the growth inhibitor of raw soy could operate through interference with the total utilization of protein or by selectively suppressing the utilization of methionine. Increasing the level of unheated soyflakes also produced parallel elevation of the growth inhibitor fraction, and thus, a diminished rate of growth was evident with high levels of protein. In a subsequent experiment it was found that the addition of methionine, but not of the whole soy protein, evoked a growth response. The authors hypothesized that possibly an inhibitor in unheated soybean flakes interfered specifically with the tissue utilization of methionine. Thus, growth inhibition was not a reflection of general interference with the availability of the whole protein, but of decreased utilization of methionine.

The specific effect exerted by the antitryptic factor has not yet been identified. A relatively high activity of intestinal enzymes with a concomitant depletion of pancreatic enzymes was seen in rats on raw soy diets when compared to others fed heated soy (Lyman and Lepkovsky, 1957). It was postulated that excessive fecal losses of enzymes may cause a partial deficiency of the most limiting amino acids (Haines and Lyman, 1961). Since methionine has been established as the first limiting amino acid of soy protein (Hayward and Hafner, 1941), fecal excretions of methionine and

nitrogen of animals fed heated or unheated soybean products were compared (Kwong, et al., 1962). A decrease in absorption of nitrogen and methionine from unheated soyflake diets was observed. However, when absorption was expressed as a ratio of the percent methionine absorbed to the percent nitrogen absorbed, a value of unity was found for diets of unheated and heated soybean products, isolated soybean protein, and casein. Thus, the low nutritive value of unheated soy cannot be attributed to elevated fecal losses of methionine.

A relatively slower stomach emptying time with raw as compared to heated soy was seen by deMuelenaere (1964). This same effect could be achieved by incorporating raw versus heated trypsin inhibitor into diets. The author suggested that the slow gastric evacuation may possibly be responsible for the decreased consumption of raw soybean products. Fractionation of intestinal contents revealed a relatively high insoluble nitrogen fraction in animals fed raw soybean products, which could originate from mucosal debris or an insoluble fraction of raw soybean. Since raw trypsin inhibitor factor and raw soybean had been found more soluble than their respective heated products, deMuelenaere presumed that the increased insoluble nitrogen fraction was due to increased mucosal debris. This suggested that the difference in nutritive value of raw and heated soy was related to the extra demand for protein for regeneration of epithelial tissue.

Apart from the effect of trypsin inhibitor on trypsin, another consequence was seen. Feeding unheated soybean or heated soybean with a single oral dose of crystalline soybean trypsin inhibitor to rats increased the amount of expired $^{14}CO_2$ derived from labelled methionine. The increased

oxidation of labelled methionine was suppressed by feeding supplementary cystine (Kwong and Barnes, 1963). The results suggested that the trypsin inhibitor blocked the utilization of cystine for protein synthesis. On the other hand, Frost and Mann (1966) observed that the feeding of raw trypsin inhibitor, of added cystine, or of raw soybean product stimulated the oxidation of labelled methionine. These authors suggested that trypsin inhibitor may affect the conversion of methionine to cystine through cystathionine. Two possible mechanisms were postulated: 1) that the trypsin inhibitor may prevent cystine incorporation into protein thus increasing the cystine pool which represses cystathionine synthetase, or 2) that it may inhibit cystathionine synthetase directly.

However, even if the growth inhibition factor of soy was not inactivated the nutritive value of soy could be made comparable to casein by supplementation of 16% soy with 0.2% methionine or cystine. The supplementation with extra choline or cyanocobalamin was without effect (Henry, et al., 1961). Soy protein also compared favorably to other proteins in the promotion of growth. Young rats fed 17% soy protein achieved the same weight gains as those on 8% egg albumen or 16% fish meal (Muramatsu and Ashida, 1963).

METHODS AND PROCEDURES

In the present study, animals were observed in 3 experiments with certain variations in diets and procedures.

Selection and care of animals

Rats obtained from a commercial firm[1] were used in the first experiment while animals bred in this laboratory were used in the other two studies. Blocks of four littermates of female albino rats, weighing between 45 and 60 g were taken at an age of approximately 21 days. Each animal was placed in a separate wire mesh cage and received its respective diet and distilled water ad libitum for approximately 28 days.[2]

Weekly food allotments of 100 g portions of diet were weighed. Each rat was fed from a designated jar, and at the end of the week, food intake was estimated by accounting for the food left and spilled.

Three times weekly rats were weighed and given supplements of cod liver oil[3] and water soluble-vitamins. A weekly dose of alpha tocopherol was provided.

Preparation of experimental diets

The composition of the diets is found in Tables 1, 2, and 3. The experimental diets were prepared in 5 or 10 kg quantities and stored at

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[1]Simonsen Laboratories, White Bear Lake, Minnesota.

[2]Rats were received in lots of 20 each in the first experiment. Since it was not possible to complete analyses which had to be carried out on the day of autopsy for more than 4 rats, it was necessary to vary the experimental period from 28 days for blocks of 4 rats. Experimental periods were 26, 27, 28, 29, or 30 days.

[3]E. R. Squibb and Sons, New York, N.Y. Vitamin assay per g: 1700 USP units Vitamin A and 170 units Vitamin D.

Table 1. Composition of diets - Experiment 1

Dietary component	PF	SM 7.5	SM 15	S4M 7.5	S4M 15	LM 7.5	LM 15	L 7.5	L 15
	g	g	g	g	g	g	g	g	g
Alpha (soy) protein[a]	-	8.4	16.7	8.0	16.0	-	-	-	-
Lactalbumin[b]	-	-	-	-	-	9.2	18.4	9.5	19.1
dl methionine[b]	-	.1	.2	.4	.8	.28	.55	-	-
Corn dextrin[c]	76	67.5	59.1	67.6	59.2	66.6	57.1	66.5	56.9
Vegetable shortening[d]	20	20	20	20	20	20	20	20	20
Salt mixture[e]	4	4	4	4	4	4	4	4	4

[a]Nutritional Giochemical Corporation, Cleveland, Ohio.

[b]General Biochemicals Inc., Chagrin Falls, Ohio.

[c]Fisher Scientific Fairlawn, New Jersey.

[d]Crisco, Procter and Gamble, Cincinnati, Ohio.

[e]Hawk and Oser salt mixture, General Biochemicals, Chagrin Falls, Ohio.

Table 2. Composition of diets - Experiment 2

Dietary component	S 3.75	S 7.5	S 15	SM 3.75	SM 7.5	SM 15
Alpha (soy) protein[a]	4.2	8.5	16.9	4.2	8.4	16.7
d-l methionine[b]	-	-	-	.05	0.1	0.2
Corn dextrin[c]	71.8	67.5	59.1	71.7	65.5	59.1
Vegetable shortening[d]	20	20	20	20	20	20
Salt mixture[b]	4	4	4	4	4	4

[a]Nutritional Biochemical Corporation, Cleveland, Ohio.

[b]General Biochemical Inc., Chagrin Falls, Ohio.

[c]Fisher Scientific, Fairlawn, New Jersey.

[d]Crisco, Procter and Gamble, Cincinnati, Ohio.

-20°C. Weekly rations were withdrawn from this supply and kept at 4°C.

The stock ration fed to control animals was prepared twice a week (Table 4). This preparation was supplemented with approximately 15 g lean ground meat, 30 g carrots and 10 g cabbage weekly.

Preparation of vitamin supplements

The composition of the water-soluble vitamin mixture is listed in Table 5. The vitamins were dissolved in 20% ethanol and kept at 4°C. The solution was of such a concentration that 2 ml 3 times a week provided the amounts listed in Table 5.

Concentrated alpha-tocopherol acetate was diluted with cottonseed oil

Table 3. Composition of diets - Experiment 3

Dietary component	S 3.75	S 7.5	S 15	S 225	S 30	SM 3.75	SM 7.5	SM 15	SM 225	SM 30
Alpha (soy) protein[a]	4.2	8.5	16.9	25.4	33.8	4.17	8.3	16.7	25.0	33.4
d-l methionine[b]	-	-	-	-	-	.05	.1	.2	.3	.4
Dextrin[b]	69.8	65.5	57.1	48.6	40.2	69.8	65.6	57.1	48.7	40.2
Vegetable shortening[c]	20	20	20	20	20	20	20	20	20	20
Salt mixture[b]	4	4	4	4	4	4	4	4	4	4
Non-nutritive fiber[b]	2	2	2	2	2	2	2	2	2	2

[a]Nutritional Biochemical Corporation.

[b]General Biochemical Inc., Chagrin Falls, Ohio.

[c]Crisco, Procter and Gamble, Cincinnati, Ohio.

Table 4. Composition of modified Steenbock XV diet

Ingredients	Percent
Cornmeal[a]	42.1
Skim milk[b]	18.4
Linseed meal[c]	12.0
Wheat germ[d]	7.5
Brewers yeast[a]	7.1
Casein (crude)[e]	3.8
Alfalfa meal[f]	1.5
NaCl (iodized salt)	0.4
Calcium carbonate[g] + trace elements[h]	0.4
Yeast (irrad.)[i] + calcium pantothenate[j]	0.4
Cottonseed oil[k]	6.4
Total	100.0

[a]General Biochemicals, Inc., Chagrin Falls, Ohio.

[b]Des Moines Coop Dairy, Des Moines, Iowa.

[c]Froning and Deppe Elevator, Ames, Iowa.

[d]General Mills, Minneapolis, Minnesota.

[e]The Borden Company, New York, New York.

[f]National Alfalfa Dehydrating and Milling Company, Kansas City, Missouri.

[g]Mallinckrodt, St. Louis, Missouri.

[h]Trace elements include:

KI	0.200 gm.
MnSO	0.790 gm.
$K_2Al_2(SO_4)_4$	0.245 gm.
$CuSO_4$	1.018 gm.
$CaCO_3$ to make 500 gm.	

[i]Irradiated in this laboratory. General Biochemicals, Inc., Chagrin Falls, Ohio.

[j]6 g calcium pantothenate added to 1 kilogram of irradiated yeast.

[k]Wesson Oil Sales Company, Fullerton, California.

Table 5. Composition of the water-soluble vitamin mixture

Vitamin[a]	Dosage per day	Vitamins per 2000 ml of 20% ethanol
Thiamine HCL	20.0 mcg	48.0 mg
Riboflavin	39.0 mcg	78.0 mg
Pyridoxine HCL	20.0 mcg	40.0 mg
Folic acid	20.0 mcg	40.0 mg
Calcium pantothenate	97.0 mcg	194.0 mg
Para-amino benzoic acid	97.0 mcg	194.0 mg
Vitamin B_{12}	0.2 mcg	0.4 mg[b]
Boitin	2.0 mcg	4.0 mg[c]
Niacin	64.0 mcg	128.0 mg
Inositol	2.4 mg	4.8 g
Choline HCL	4.8 mg	9.6 g

[a]All vitamins were obtained from General Biochemicals, Inc.

[b]400 mg of vitamin B_{12} in mannitol, 0.1 per cent triturated.

[c]400 mg of Biotin in dextrin, 1 per cent triturated.

so that a dose of 50 mg supplied 7 mg of the vitamin per week. 100 mg cod liver oil[1] was administered orally three times a week to supply vitamins A and D.

Animals were usually[2] autopsied on the 28th day of the experimental period. The rat was stunned and decapitated. The liver was promptly removed, trimmed of excess tissue, dipped into a chilled physiological saline solution, blotted, and weighed. A portion of less than 500 mg was taken from the tri-lobate section and assayed for FAO activity. The remainder of the liver was divided into appropriate size portions.

[1]Animals on stock ration were given cod liver oil supplement only.

[2]See footnote 2, Methods and Procedure, page 32.

These were weighed and prepared for other analysis. Weighed portions were also sealed in plastic bags, frozen in liquid nitrogen and stored in the frozen state for analysis of nitrogen, nucleic acids, and lipids.

Analytical procedures

Nitrogen determination Hepatic nitrogen concentration was determined by the method of Dumas.[1] Approximately 1 mg liver was combusted at 800°C in the presence of cuprous oxide in a closed system. The resulting mixture of gases was flushed through cupric oxide and potassium hydroxide to reduce and remove all gases but nitrogen. The volume of nitrogen gas produced, measured in a calibrated syringe, was converted to an equivalent weight with an equation based on general gas laws.

GPT GPT was assayed by a procedure described by Sigma Chemical Company, 1961 (Table 6). This enzyme transfers the amino group from alanine to alpha-ketoglutaric acid producing pyruvic acid and glutamic acid. Evidence of this reaction was based on the reduction of the product, pyruvic acid, by NADH. Since NADH has a high optical density at 340 mu and its oxidized counterpart does not, the rate of change in optical density was followed spectrophotometrically[2] at that wave length.

FAO A modification (Crenshaw, 1962) of the procedure described by Greenbaum and McLean (1953) was used to determine activity of the FAO system using a short chain fatty acid as a substrate. The amount of oxygen required by a liver homogenate for oxidation of caprylic acid was determined manometrically. In the presence of reagents and co-factors listed

[1]Coleman Model 29 Nitrogen Analyzer. Coleman Instruments, Inc., 42 Madison St., Maywood, Illinois.

[2]Beckman DU, Beckman Co., Fullerton, California.

Table 6. Assay for hepatic glutamic pyruvic transaminase: measurement of decrease in OD at 340 mu per minute

Reagent	Concentration	Volume/ reaction
NADH solution[a,b]	1mg/ml	0.2 ml
dl alanine[b]	4×10^{-1} M	0.5 ml
Lactic dehydrogenase[b]	8000 units/ml	0.1 ml
Water	-	1.8 ml
Liver homogenate	-	0.2 ml
Ketoglutarate solution[b]	1×10^{-1} M	0.2 ml

[a]Reduced form B-diphosphopyridine nucleotide (reduced nicotinamide adenine dinucleotide phosphate).

[b]Sigma Chemical Company, St. Louis, Missouri.

in Table 7 the amount of oxygen utilized by 50 mg liver was followed for 20 minutes at 32°C. The critical step of this method was to start the reaction as soon as possible. Including a 10 minute equilibration period, the first reading was always taken within 18 minutes after the rat was sacrificed.

The method described by Bremer (1962a) was used to determine the FAO using a long chain fatty acid as a substrate. The uptake of molecular oxygen for the oxidation of palmitylcarnitine by mitochondria from 250 mg liver was determined for 20 minutes at 32°C. The reagents are listed in Table 8. The palmitylcarnitine was prepared according to procedures described by Bremer (1962b).

Hepatic lipids One half gram liver was extracted with a system of solvents described by Soderhjem and Soderhjem (1949) in specially designed extraction flasks.[1] The lipid containing layers of ethanol,

[1]Mojannier extraction flask.

Table 7. Assay for Fatty Acid Oxidase: measurement of oxygen uptake by liver homogenate using octanoate as a substrate

Reagent	Concentration	Volume/reaction flask	
		Experimental (ml)	Blank (ml)
Sodium phosphate buffer[a]	1.00×10^{-2}M	0.6	0.6
Potassium chloride	2.50×10^{-2}M	0.3	0.3
Magnesium chloride	6.00×10^{-3}M	0.3	0.3
Sodium succinate[b]	1.00×10^{-3}M	0.3	0.3
Adenosine triphosphate[a]	2.00×10^{-3}M	0.3	0.3
Caprylic acid[b]	1.33×10^{-3}M	0.3	
Cytochrome C[a]	2.66×10^{-5}M	0.3	0.3
Distilled water		-	0.3
Liver homogenate	83.3 mg/ml	0.6	0.6

0.2 ml KOH and filter paper were placed in center well of reaction flasks to absorb CO_2 produced.
All reagents, except liver homogenate, were at room temperature (25°C) and adjusted to pH 7.4 before use.
Liver was homogenized in 0.25 M sucrose solution (40°C).

[a]Sigma Chemical Company, St. Louis, Missouri. (Also in Table 8)

[b]Calbiochem, Los Angeles, California. (Also in Table 8)

Table 8. Assay for Fatty Acid Oxidase: measurement of oxygen uptake by liver mitochondria using palmitylcarnitine as a substrate

Reagent	Concentration
Potassium phosphate buffer, pH 7.4	4×10^{-2}M
Magnesium chloride	2×10^{-2}M
Adenosine monophosphate[a]	1×10^{-1}M
Sodium succinate[b]	3×10^{-3}M
Bovine serum albumin[a]	3×10^{-2}M
Potassium chloride	1.5×10^{-2}M
Liver mitochondria	
10% sucrose with 0.01 M EDTA (neutralized)	
Palmitylcarnitine	

0.2 ml KOH and filter pater in center well; mitochondria prepared from 250 mg liver by centrifuging at 800 x g for 5 minutes to remove nuclei and cellular debris. Supernatant was then sedimented at 8,000 x g for 15 minutes and resuspended.

petroleum ether, and anhydrous ethyl ether were decanted into tared weighing bottles and dried at 80°C for 15½ hours. Time and temperature had been predetermined to give the minimum reproducible weight. The bottles were cooled in a desiccator for 4 hours and weighed.

RNAse The RNAse activity was determined according to the method described by Roth (1959). 300 mg liver was incubated with 1% RNA solution for 30 minutes at 37°C (Table 9). The change in optical density due to increase in nucleotide at 260 mu was read in a spectrophotometer and compared to the change produced by known amounts of purified RNAse.

Table 9. Assay for Ribonuclease, pH 7.7

Concentrations	Sample ml	Blanks ml	Standards ml
Veronal-acetate buffer, pH 7.8	0.5	0.5	0.5
1% RNA[a,b] solution	0.5	0.5	0.5
Liver homogenate	1.5	-	-
Distilled water	-	1.5	-
Standard RNAse solution[b]	-	-	1.5

[a]Ribonucleic Acid.

[b]Sigma Chemical Company, St. Louis, Missouri.

SDH In Experiments 2 and 3, SDH activity was estimated by a colorimetric method described by Muramatsu and Ashida (1961). The reagents are listed in Table 10. The determination is based on a change in color when the ring

of INT is opened by a transfer of hydrogen from SDH. The reduced formazan was extracted with butanol and the optical density determined spectrophotometrically. The wave length used was 500 mu since the peak of absorption occurs at that point. The concentration was determined by comparing the change in optical density to that of standards prepared from known amounts of INT completely reduced by sodium hydrosulfite.

In Series 1, SDH activity was estimated by procedures of Nachlas, Margulies, and Seligman (1960). The principle of the method is similar to that used in Experiments 2 and 3, except that the reduced INT was extracted in the presence of phenazine methosulfate instead of butanol.

Nucleic acids The concentration of RNA and DNA was determined by the method described by Schmidt and Thannhauser (1945) as modified by Wannemacher, et al. (1965). A homogenate containing 20% liver was washed in cold trichloroacetic acid to remove acid soluble compounds, which may interfere with the determination. The resulting precipitate was extracted of lipids with a solvent system consisting of ethanol, ether, and chloroform. After the solvents were evaporated, the residue was digested in dilute alkali. By acidification of the alkaline hydrolysate the nucleic acids were separated with RNA in the acid soluble fraction and the DNA in the insoluble fraction. The DNA containing residue was heated in 60% perchloric acid for 1 hour at 95°C to solubilize the DNA. The increase in optical density of the two solutions was determined spectrophotometrically at 260 mu. The optical density due to the aromatic amino acids was corrected for by subtracting a reading taken at 290 mu. The concentration of RNA and DNA was calculated from a standard prepared by treating known

Table 10. Assay for Succinic dehydrogenase

Reagent	Concentration	ml sample	ml std.	ml sample blank	ml std. blank
Sodium Succinate[a]	5.00×10^{-1}M	0.5 ml	0.5 ml	-	0.5 ml
Sodium Fumarate[b]	1.00×10^{-1}M	-	-	0.5 ml	-
Phosphate buffer, pH 7.2	1.00×10^{-1}M	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Liver homogenate	2 mg/ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Iodonitrotetrazolium violet[d]	2 mg/ml	0.5 ml	0.5 ml[c]	0.5 ml	-
Distilled water	-	-	-	-	0.5 ml

[a]Fisher Laboratory Chemicals, Fairlawn, New Jersey.

[b]Calbiochem, Los Angeles, California.

[c]For standard curve, INT solution was diluted such that concentrations of 0.02, 0.04, 0.06 mg/ml. were used.

[d]2 p-iodophenyl-3-p-nitrophenyl-5-phenyl tetrazoluim chloride (INT) Sigma Chemical Company, St. Louis, Missouri, (INT also known as formazan).

amounts of commercial nucleic acids[1] in the same way as the liver.

Statistical analysis Group means were compared by Duncan's Test (Steel and Torrie, 1960) and standard errors of the mean were computed (Snedecor, 1956) primarily to compare experimental and control values.

In Experiment 3, the slope of a line which best described the response of each parameter to stepwise increases in protein intake was computed by standard regression equations at the ISU Computation Center. The slopes of the lines were compared by the F test (Snedecor, 1956).

[1]Yeast RNA and thymus DNA. Sigma Chemical Company, St. Louis, Missouri.

RESULTS

The present study was conducted in three sections, Experiments 1, 2, and 3. The first experiment was designed to assess the influences of methionine supplementation of 7.5 and 15% protein diets on selected parameters related to hepatic metabolism. Soy protein with 0.2% (low) and 0.8% (high) methionine and lactalbumin with or without addition of the amino acid were the variables investigated. Since alterations in diet failed to demonstrate differences in most responses measured, another experiment was conducted using 3.75, 7.5 and 15% soy protein with or without a low methionine supplement. Results of Experiment 2 indicated further pertinent information could be obtained if amounts of dietary protein in excess of 15% were used. Thus, in the last experiment, dietary variables were expanded to include inadequate as well as liberal levels of protein intake, ranging from 3.75 to 30% soy protein, with or without added methionine.

In all experiments, high and low protein control diets were used for reference points. A laboratory ration containing 28% protein served as the high-protein stock control. In Experiment 1, the low protein reference was a protein-free ration, but animals fed this diet were moribund. Thus, for Experiments 2 and 3, low protein controls were given diets of 3.75% soy protein with or without methionine.

In reporting the data of this investigation, emphasis will be placed on those obtained in Experiment 3 because relatively more dietary variables were included and more parameters were measured. Having an extensive

Table 11. Mean values for body and liver weights of young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiments 1, 2, 3

Exp. no.	Diet	No. rat	Body weight for 28 days			Liver weight	Liver wt.
			Initial	Final	Gain		100 g body
			g	g	g	g	weight
1	PF	10	68	45	-23	2.06	4.6 ± .2[a]
	L 7.5	10	69	133	64	5.83	4.4 ± .2
	L 15	10	68	165	97	7.68	4.6 ± .2
	LM 7.5	10	69	137	68	6.34	4.6 ± .1
	LM 15	10	67	165	98	6.93	4.2 ± .1
	SM 7.5	10	67	78	11	3.62	4.7 ± .2
	SM 15	10	66	142	76	5.85	4.1 ± .2
	S4M 7.5	10	68	86	18	4.25	5.0 ± .3
	S4M 15	10	63	143	80	6.45	4.5 ± .1
	Stock	10	63	167	101	7.29	4.3 ± .2
2	S 3.75	10	57	47	-10	2.27	4.8 ± .2
	S 7.5	10	57	54	-3	2.65	4.9 ± .1
	S 15	10	56	74	18	3.64	4.9 ± .2
	SM 3.75	10	55	50	-5	2.32	4.6 ± .1
	SM 7.5	10	56	75	19	3.39	4.6 ± .2
	SM 15	10	54	114	60	4.27	3.7 ± .1
	Stock	10	54	177	123	8.38	4.8 ± .2
3	S 3.75	6	55	45	-10	2.12	4.6 ± .2
	S 7.5	9	54	49	-5	2.12	4.6 ± .1
	S 15	9	52	73	21	3.09	4.2 ± .1
	S 22.5	9	54	88	33	3.56	4.0 ± .1
	S 30	9	53	101	49	4.07	4.1 ± .1
	SM 3.75	6	55	48	-8	2.12	4.5 ± .1
	SM 7.5	9	54	64	10	2.75	4.2 ± .2
	SM 15	9	52	120	68	4.64	3.8 ± .1
	SM 22.5	9	54	133	79	4.67	3.6 ± .1
	SM 30	9	52	130	78	5.00	3.9 ± .1
	Stock	12	52	153	101	6.61	4.4 ± .1

[a]Standard error of the mean

sequence of protein levels allowed for analysis by regression equations and determination of correlation coefficients. Results of Experiments 1 and 2 will be treated as preliminary data.

A pattern will generally be followed for clarity in presenting results of Experiment 3. Responses to varying protein intake in the unsupplemented (diets S) and the supplemented series (diets SM) will be reported in that order with statistical evaluation by regression equations in each series. Subsequently, the effect of methionine at each level of protein will be considered by comparison of isonitrogenous S and SM diets. 'F' values for the significance of treatment and litter effects are given in tabular forms. Differences in groups means were tested for significance with Duncan's test (Steel and Torrie, 1960).

Body and liver weights

All experimental diets, except diet S 7.5, promoted some growth (Table 11, Figures 1, 2). As was expected, changes in body weight were related to increases in quantities of soy protein ($P<0.01$) (Tables 11, 12). This was particularly evident in the unsupplemented series. Groups S 15 and S 30 weighed 150 and 200% times that of group S7.5 (Table 13). Body weights increased rapidly with a change from 7.5 to 15% supplemented protein, but the growth curve showed a plateau from this point, since further additions of supplemented protein produced no significant changes.

Diets of soy protein with methionine were associated with significantly greater weight gains than unsupplemented diets at each level of protein studied ($P<0.01$) (Tables 11, 12). Results from Experiment 1 indicated that variations in high or low levels of methionine supplement did

Table 12. F values for regression equations for body weight, liver weight and food intake - Experiment 3

Source of variation	d.f.	Body wt. g	Liver wt. g	Food intake g
Regression	24	25.88**	10.02**	6.89**
Treatments	7	83.71**	29.48**	18.09**
Methionine (M)	1	13.55**	7.26**	5.68**
Protein-linear (A)	1	16.35**	10.07**	7.88**
Protein-quadratic (B)	1	-6.52**	-3.51**	-3.60**
Protein-cubic (C)	1	2.04	2.09	.86
Interaction MA	1	-1.01	-.33	2.44
Interaction MB	1	4.47**	1.89	2.52*
Interaction MC	1	-1.71	-1.25	-2.89
Litter	17	1.53	1.75	1.45
Error	47			

*Significant at 5% level.

**Significant at 1% level.

did not affect the growth response (Table 11).

Weights of animals fed comparable isonitrogenous soy protein diets were similar in all experiments (Table 11). An exception was that group SM 15 in Experiment 1 weighed more than those fed 15% protein in the other two experiments. The relatively high initial weight of animals in Experiment 1 may explain why group SM 15 grew more rapidly. However, when the amount of dietary protein was marginal, relatively high initial body weights did not appear to offer any advantage since final body weights of group SM 7.5 in the 3 experiments were not different. Part of the difference in initial body weights of animals could be attributed to the fact that animals in Experiment 1 were obtained from a different colony than those of other experiments.

Figure 1. Mean final body weights and mean liver weights of rats fed varying quantities of soy protein without methionine (diets S) - Experiment 3.

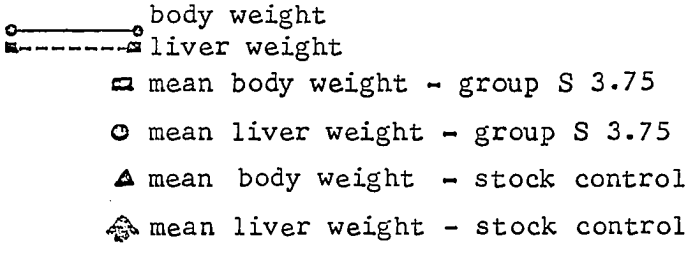
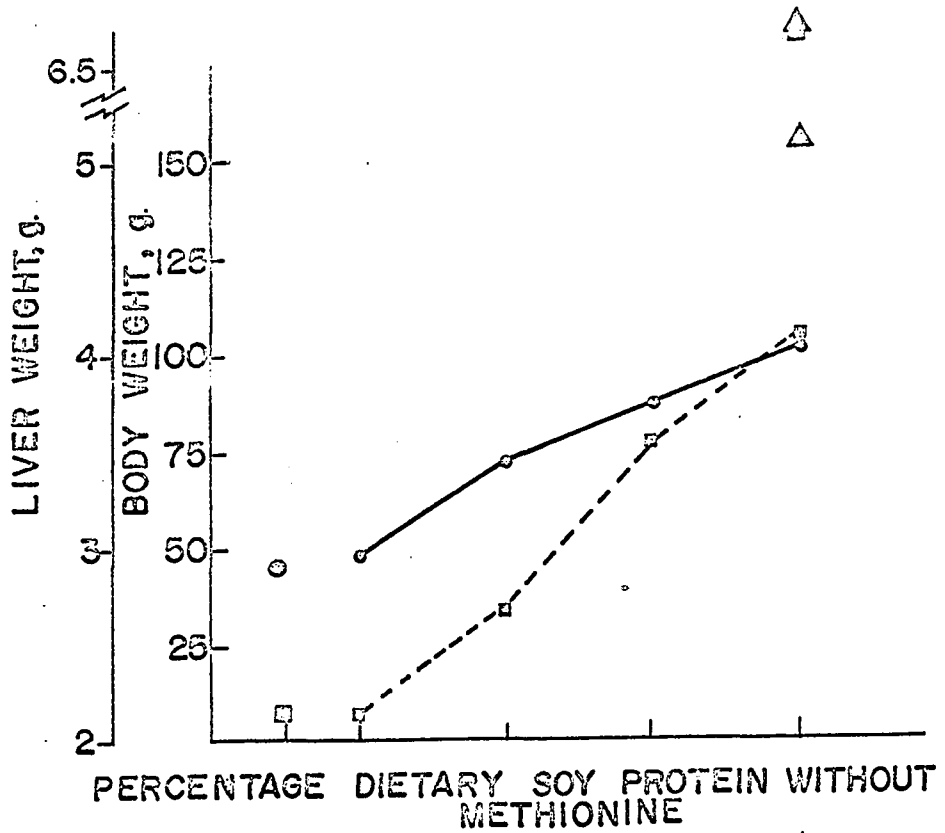


Figure 2. Mean final body weights and mean liver weights of rats fed graded quantities of soy protein with methionine (diets SM) - Experiment 3.


LIVER WEIGHT, g.
BODY WEIGHT, g.
6.5
5
4
3
2
150
125
100
75
50
25
PERCENTAGE DIETARY SOY PROTEIN WITHOUT METHIONINE

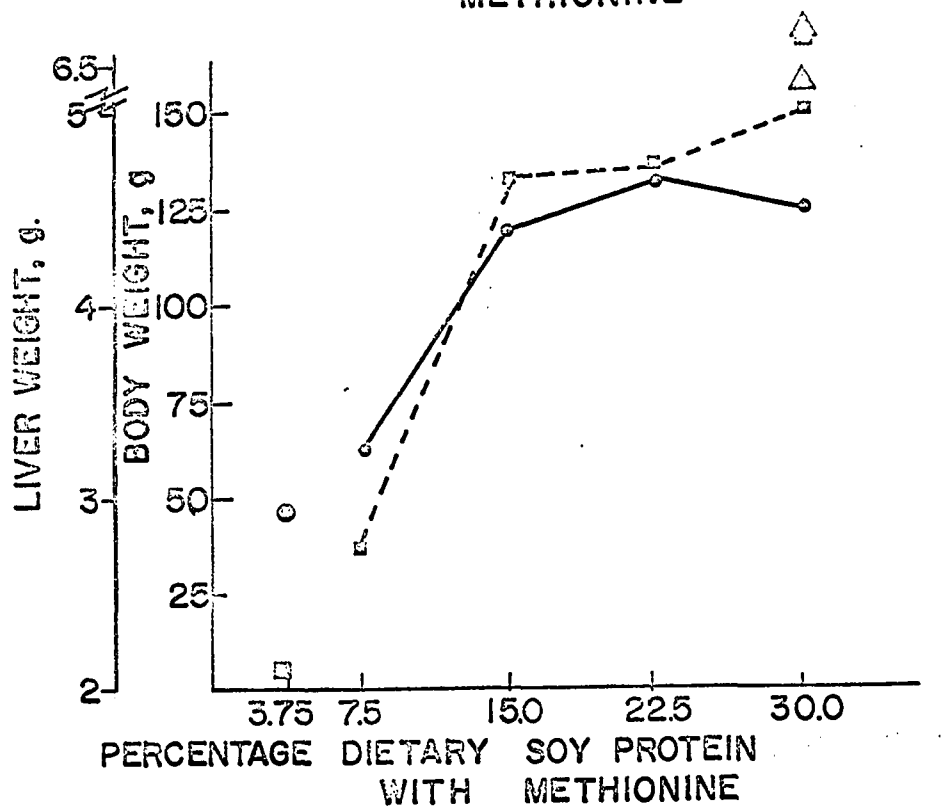

LIVER WEIGHT, g.
BODY WEIGHT, g
6.5
5
4
3
2
150
125
100
75
50
25
3.75
7.5
15.0
22.5
30.0
PERCENTAGE DIETARY SOY PROTEIN WITH METHIONINE

Table 13. Results of Duncan's Test for the effect of varying levels of protein on body weight, liver weight, and food intake[a] - Experiment 3

Series	% protein	7.5	15.0	22.5	30.0
S	Body wt	48	71	88	101
	P<.01	-----	-----	-----	-----
	P<.05	-----	-----	-----	-----
SM	Body wt	65	122	129	130
	P<.01	-----	----------		
	P<.05	-----	----------		
S	Liver wt.	2.12	3.09	3.56	4.07
	P<.01	-----	----------		
	P<.05	-----	----------	----------	
SM	Liver wt.	2.75	4.64	4.67	5.00
	P<.01	-----	----------		
	P<.05	-----	----------		
S	Food intake	143	162	211	227
	P<.01	----------		----------	
	P<.05	----------		----------	
SM	Food intake.	170	254	241	234
	P<.01	-----	----------		
	P<.05	-----	----------		

[a]Any two means not underscored by the same line are significantly different. Comparison may be between groups of means.

Table 14. Results of Duncan's Test for the effect of methionine on body weight, liver weight, and food intake[a] - Experiment 3

% protein	7.5		15.0		22.5		30.0	
Group	S	SM	S	SM	S	SM	S	SM
Body wt	48	65	71	122	88	129	101	130
P <.01	----	----	----	----	----	----	----	----
P <.05	____	____	____	____	____	____	____	____
Liver wt.	2.12	2.75	3.09	4.64	3.56	4.67	4.07	5.00
P <.01	----------		----	----	----	----	----	----
P <.05	____	____	____	____	____	____	____	____
Food intake	143	170	162	254	211	241	227	234
P <.01	----------		----	---	----------		----------	
P <.05	_____		____	___	_____		_____	

[a]Any two means not underscored by the same line are significantly different.

The growth of animals on experimental soy diets, even at high levels of protein intake was not comparable to that of rats on stock diets (Table 11). When 15% lactalbumin diets were fed in Experiment 1, body weights were similar to those of stock controls. The addition of methionine to 7.5% soy protein promoted some growth whereas the supplemented 3.75% protein diet was associated with weight loss in Experiment 2.

Mean liver weights of experimental groups in Experiment 3 ranged from 2.12 to 5.00 g (Tables 11, 13). That alterations in liver size

paralleled changes in body size is illustrated in Figures 1, 2. Since a nearly perfect correlation (r=.94) between liver and body weight had been found ($P < 0.01$), a ratio of these 2 parameters was computed. The ratio of liver to body weight of group S 7.5 was greater than that of groups S 22.5 and S 30 (Table 11). This finding may be related to a relatively large amount of hepatic fat in proportion to body size of group S 7.5 in Experiment 3.

In groups fed supplemented soy protein, liver weights were essentially proportional to body size except in group SM 22.5 (Table 11). The value for body weight of group SM 22.5 exceeded those of groups SM 15 and 30 whereas liver weights in the comparison showed reversed tendencies. Although these differences were not significant, a ratio of the factors apparently magnified the variations.

In Experiment 3, liver to body weight ratios tended to decrease as dietary protein was increased. The proportion of liver to body weights of low protein and stock controls was not different from that of experimental groups.

Liver nitrogen

Changes in nitrogen concentration paralleled stepwise increases in dietary protein ($P < 0.01$) (Tables 15, 16a, Figure 3). Means of groups S 7.5 and S 15 were similar (2.14 and 2.30% respectively) ($P < 0.05$) and less than those for groups S 22.5 and S 30 (2.60 and 2.74% respectively) (Table 17). Values associated with low levels of supplemented protein were significantly increased by elevating the percent of supplemented soy protein from 15 to 22.5. An additional increment of supplemented protein to 30%

Table 15. Hepatic nitrogen and hepatic lipids in young female rats fed graded quantities of soy protein with or without methionine supplement for 28 days - Experiment 3

		Percent dietary soy protein					
	Diet	3.75	7.5	15.0	22.5	30	Stock
Percent nitrogen wet weight	S	2.29	2.14	2.30	2.60	2.74	3.21
	SM	2.64	2.61	2.49	3.06	3.48	
Percent lipid wet weight	S	10.7	10.9	13.8	9.3	6.4	
	SM	5.8	7.4	7.4	5.3	5.1	4.2
Percent nitrogen fat free basis	S	2.53	2.39	2.67	2.86	2.93	
	SM	2.85	2.82	2.69	3.24	3.67	3.35
mg nitrogen per 100 g body weight	S	1.06	.93	1.00	1.05	1.19	
	SM	1.15	1.09	.94	1.10	1.33	1.43

Table 16a. F values for regression equations for hepatic nitrogen values

Source of variation	d.f.	Nitrogen g
Regression	24	3.85**
Treatments	7	10.64**
Methionine (M)	1	4.83**
Protein-linear (A)	1	6.16**
Protein-quadratic (B)	1	1.35
Protein-cubic (C)	1	-1.34
Interaction MA	1	-1.25
Interaction MB	1	-1.46
Interaction MC	1	.66
Litter	17	1.18
Error	47	

*Significant at 5% level.

**Significant at 1% level.

Table 16b. Results of Duncan's test for effect of methionine on hepatic nitrogen[a] - Experiment 3

Percent dietary protein	7.5		15.0		22.5		30.0	
Group	S	SM	S	SM	S	SM	S	SM
Percent hepatic nitrogen	2.14	2.61	2.30	2.49	2.60	3.06	2.74	3.48
P< 0.01	----------		---------		---------		----------	
P< 0.05	____	____	_____		____	____	_____	____

[a]Any two means not underscored by the same line are significantly different.

Table 17. Results of Duncan's test for effect of varying levels of protein on hepatic nitrogen[a] - Experiment 3

Group	S 7.5	S 15.0	S 22.5	S 30.0
Percent hepatic nitrogen	2.14	2.30	2.60	2.74
P< 0.01	----------			
P< 0.05	_____		_____	

Group	SM 7.5	SM 15.0	SM 22.5	SM 30.0
Percent hepatic nitrogen	2.61	2.49	3.06	3.48
P< 0.01	----------			
P< 0.05	_____		____	____

[a]Any means not underscored by the same line are significantly different.

Figure 3. Mean per cent hepatic nitrogen wet weight in young female rats fed varying quantities of soy protein, with or without methionine - Experiment 3.

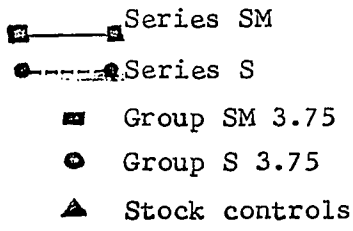
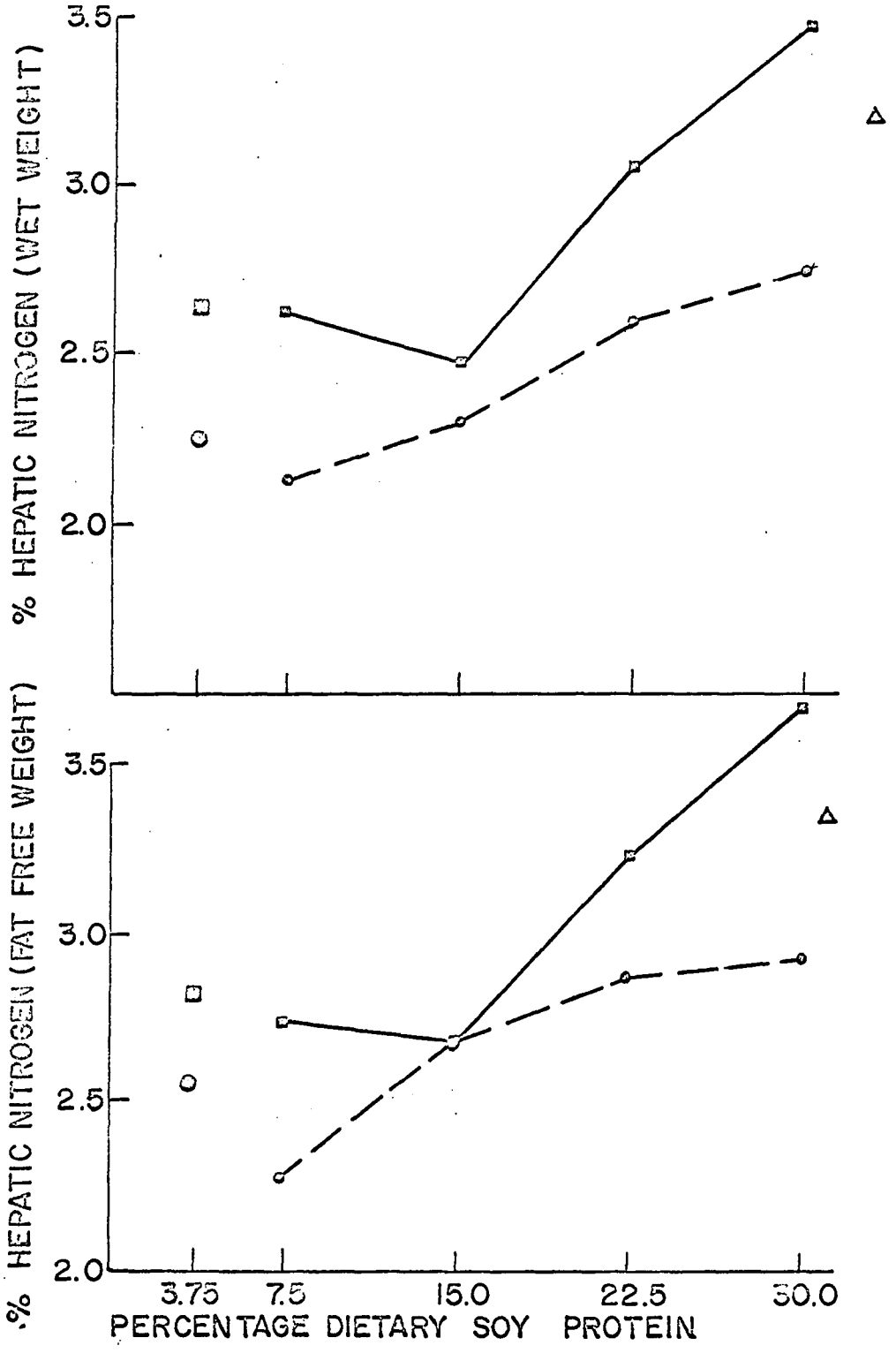


Figure 4. Mean per cent hepatic nitrogen fat free weight in young female rats fed varying quantities of soy protein, with or without methionine - Experiment 3.


% HEPATIC NITROGEN (WET WEIGHT)
% HEPATIC NITROGEN (FAT FREE WEIGHT)
3.5
3.0
2.5
2.0
3.75
7.5
15.0
22.5
30.0
PERCENTAGE DIETARY SOY PROTEIN

produced a further significant rise in nitrogen concentration. Mean value for this group was 3.48% and exceeded that found for stock controls (3.21%), but the difference was statistically insignificant.

The addition of methionine caused a significant increase in means of hepatic nitrogen at most levels of dietary protein investigated (Tables 15, 16a, 16b). Data from Experiment 3 showed a significant relationship between the addition of methionine and the elevation of nitrogen concentration ($P < 0.01$).

It appears that the level of supplementation used here, namely, 0.2% methionine for every 15% soy protein, produced the maximum effect, since in Experiment 1, a fourfold increase of the supplement had failed to produce further changes in liver nitrogen concentration (Table 18).

Generally, the lowest nitrogen concentrations were observed in livers which showed the highest lipid values. The quantity of hepatic fat varied from 4 to 14% of wet weight (Table 15). In an attempt to estimate the functional tissue of the liver and to assess the dilution of nitrogen concentration by lipid infiltration, weight of fat-free tissue was computed (Table 15). Since differences in nitrogen values were still apparent on a fat free basis, the relatively low concentrations of hepatic nitrogen observed with unsupplemented diets had not been solely caused by dilution of active tissue with lipids (Figure 4).

Differences in nitrogen concentrations among groups receiving comparable diets in the 3 experiments were seen, but values in animals fed stock diets appeared relatively constant throughout the entire study (Table 18). This observation would indicate that differences between

Table 18. Percent hepatic nitrogen and lipids in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiments 1, 2, 3

Experiment	Diet	No. rats	Hepatic nitrogen %	Hepatic lipid %
1	PF	10	2.39	10.6 ± 1.2[a]
	L 7.5	10	2.90	11.8 ± 1.5
	L 15	10	3.15	10.3 ± 1.2
	LM 7.5	10	2.53	6.8 ± .4
	LM 15	10	2.79	6.0 ± .3
	SM 7.5	10	2.66	10.5 ± 1.3
	SM 15	10	3.19	7.4 ± 0.9
	S4M 7.5	10	2.54	8.1 ± 0.9
	S4M 15	10	3.01	5.5 ± 0.5
	Stock	10	3.17	4.7 ± 0.3
2	S 3.75	10	2.55	14.2 ± 1.5
	S 7.5	10	2.79	17.0 ± 0.7
	S 15	10	2.94	13.3 ± 1.4
	SM 3.75	10	2.81	12.0 ± 1.8
	SM 7.5	10	3.01	9.7 ± 1.2
	SM 15	10	3.26	7.8 ± 0.8
	Stock	10	3.25	4.6 ± 0.5
3	S 3.75	6	2.29	10.7 ± 1.1
	S 7.5	9	2.14	10.9 ± 1.4
	S 15	9	2.30	13.8 ± 1.3
	S 22.5	9	2.60	9.3 ± 1.2
	S 30	9	2.74	6.4 ± 0.6
	SM 3.75	6	2.64	5.8 ± 1.4
	SM 7.5	9	2.61	7.4 ± 1.1
	SM 15	9	2.49	7.4 ± 0.7
	SM 22.5	9	3.06	5.3 ± 0.5
	SM 30	9	3.48	5.1 ± 0.4
	Stock	12	3.21	4.2 ± 0.9

[a]Standard error of the mean

experiments were probably not due to variations in methodology, but rather to deviations in responses by animals of different experiments to similar experimental treatments. However, in spite of variations in magnitudes between studies, the pattern of response to varying quantity and quality of protein appeared similar in the 3 experiments.

With most dietary treatments, the total hepatic nitrogen content changed as the body weights were altered (Table 15). Ratios of nitrogen per 100 g body weight were all close to 1 except for groups SM 30 and stock controls. These results were not surprising since the nitrogen concentration of group SM 30 exceeded that of all other experimental groups, while body weights had been similar to those of groups SM 22.5 and SM 15. The relatively high ratio of nitrogen to body weight of the stock control group reflected large liver sizes.

Hepatic lipids

The degree of hepatic lipid infiltration appears to be influenced by the quantity and quality of the protein (Tables 15, 18, 19). In the series of animals receiving soy protein without additional methionine, the highest concentration of liver lipids was found in group S 15 with an average of 13.8% (Tables 15, 18, 20; Figure 5). Means for both groups S 22.5 and S 30 were significantly lower but not statistically different from each other (9.3 and 6.4% respectively). Only the value associated with diet S 30 approached that seen in stock controls (4.2%). This suggested that the lipid values were progressively reduced as stepwise increments of protein were made to diet S 15.

Table 19. F values for regression equations for hepatic lipid values - Experiment 3

Source of variation	d.f.	Lipid g
Regression	24	3.18*
Treatments	7	8.82**
Methionine (M)	1	5.80**
Protein-linear (A)	1	-4.56**
Protein-quadratic (B)	1	-2.27*
Protein-cubic (C)	1	-2.24*
Interaction (MA)	1	-1.53
Interaction (MB)	1	-2.14
Interaction (MC)	1	-0.94
Litter	17	.64
Error	47	

*Significant at 5% level.

**Significant at 1% level.

Table 20. Results of Duncan's Test for the effect of varying levels of protein on lipid[a] - Experiment 3

Series	% protein	15.0	7.5	22.5	30.0
S	Lipid/g	13.8	10.9	9.3	6.4
	P< .01	----------	----------	----------	
	P< .05	_____	_____	_____	_____
SM	Lipid/g	7.4	7.4	5.3	5.1
	P< .01	----------			
	P< .05	_____			

[a]Any means not underscored by the same line are significantly different.

Table 21. Results of Duncan's Test for the effect of methionine on lipid[a] - Experiment 3

Group	% protein	7.5		15.0		22.5		30.0	
		S	SM	S	SM	S	SM	S	SM
Lipid/g		10.9	7.4	13.8	7.4	9.3	5.3	6.4	5.1
	P< .01	----------		----	----	----------		----------	
	P< .05	____	____	____	____	____	____	_____	

[a]Any two means underscored by the same line are not significantly different.

Figure 5. Mean percent hepatic lipids of rats fed varying quantities of soy protein without methionine (diets S) - Experiment 3.

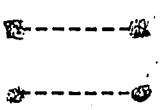
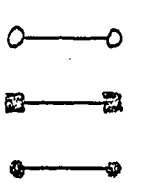
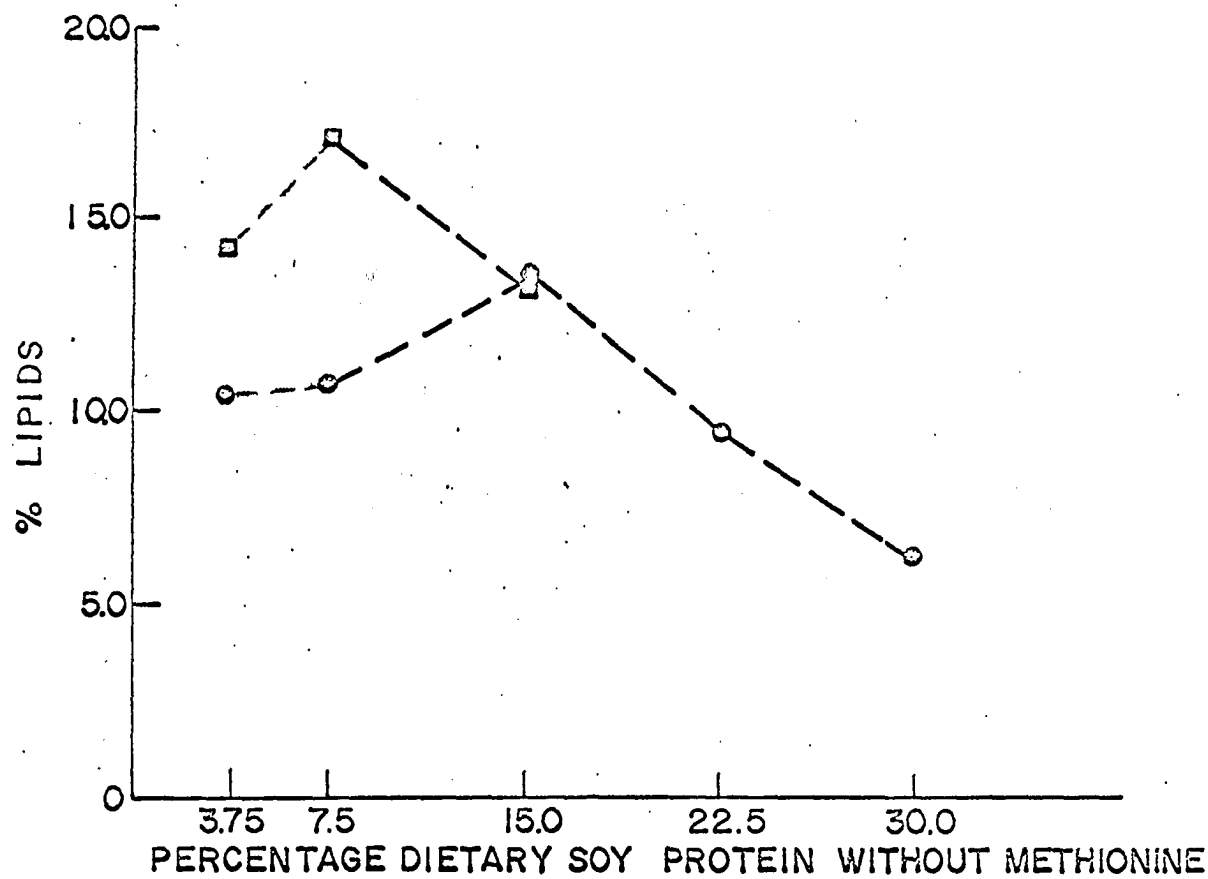
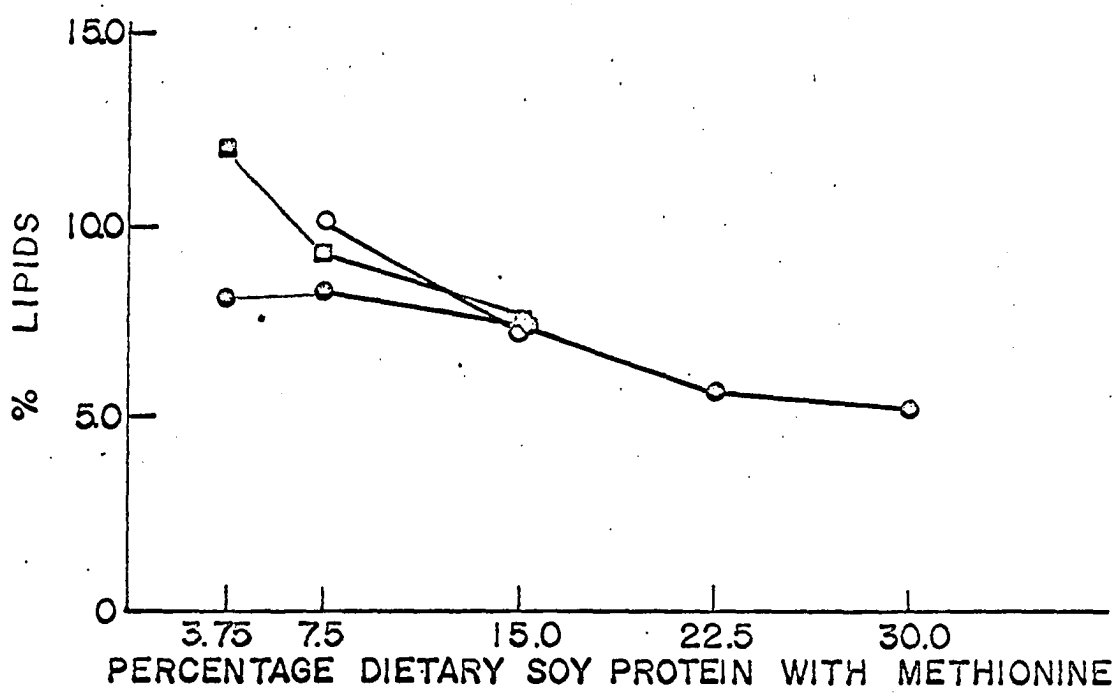


Figure 6. Mean per cent hepatic lipids of rats fed varying quantities of soy protein with methionine (diets SM).- Experiment 3.




20.0
15.0
10.0
5.0
0
% LIPIDS
3.75
7.5
15.0
22.5
30.0
PERCENTAGE DIETARY SOY PROTEIN WITHOUT METHIONINE


15.0
10.0
5.0
0
% LIPIDS
3.75
7.5
15.0
22.5
30.0
PERCENTAGE DIETARY SOY PROTEIN WITH METHIONINE

A narrow range of hepatic lipid values, 5.1 to 7.4%, was found in rats fed supplemented soy protein diets (Tables 15, 18; Figure 6). Also, diets with added methionine were associated with lower lipid values than isonitrogenous unsupplemented diets. The lipotropic effect of additional methionine was apparent at all but the highest level of protein tested (Table 21). Apparently, 30% soy protein supplied enough methionine to mask any effect which additional amounts of this amino acid might have had on liver lipids. These observations confirmed previous reports which indicated that the lipotropic effect of methionine was especially evident when concentrations of dietary protein were low. Mean lipid values of comparable groups in the different experiment receiving diets S 15, SM 15, and stock controls agreed fairly well (Tables 15, 18). In contrast, groups receiving less soy protein than 15% showed varied responses in lipid concentration.

In Experiment 1, mean lipid values for animals maintained on 7.5 or 15% lactalbumin were not different (11.8 and 10.3% respectively) and were depressed to the same extent by the addition of methionine to the diet (6.8 and 6.0% respectively) (Table 18). In contrast, mean lipid concentrations were less in animals receiving 15% soy protein than in those on 7.5% soy protein. The soy protein diets used in Experiment 1 contained either a high or low methionine supplement, but the level of supplementation did not appear to affect the lipid concentration. Animals on the protein free ration had 10.6% lipids in livers.

Nucleic acids

The hepatic DNA as well as the hepatic RNA content was determined in Experiment 3. The concentrations of these nucleic acids appeared to be unchanged by dietary treatment, but because of variations in liver sizes, differences in total hepatic DNA and RNA values were seen.

Mean DNA values ranged from 1.22 to 1.54 mg per g liver (Tables 22, 23, 24) and were not significantly altered by dietary variations. In this study, the extreme mean values (1.22 and 1.54) were seen in two adjacent groups, SM 15 and SM 22.5. When other liver components for these two groups were expressed relative to DNA, significant differences in ratios occurred.

RNA per g liver was unaltered by increasing the protein content of the diets or by supplementing with methionine (Table 22, Figures 7, 8, 9). Since at the cellular level, RNA is intimately associated with protein biosynthesis as well as DNA, investigators have found it informative to consider hepatic RNA relative to nitrogen and DNA. For this reason, RNA data were expressed on the basis of nitrogen (RNA/N) and DNA (RNA/DNA). When this was done, RNA concentration relative to nitrogen decreased as graded amounts of soy protein were incorporated into diets ($P < 0.01$) Table 25), reflecting increases in nitrogen concentration under the same conditions. The RNA/DNA ratios were not modified by dietary alterations (Tables 26, 27), since both parameters were relatively refractive to changes in diets. However, differences in mean RNA/DNA or RNA/N ratios were observed since in computing ratios, insignificant variations of one parameter were magnified by opposing changes of the second parameter.

Table 22. Mean hepatic nucleic acid values of young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiment 3

Experiment	No. rats	Diet	mg DNA / g liver	mg RNA / g liver	mg RNA / mg nitrogen	mg RNA / mg DNA
3	6	S 3.75	1.30±.10[a]	12.53±.98	.57±.06	10.26±1.40
	9	S 7.5	1.25±.08	12.29±.58	.59±.05	10.24±0.94
	9	S 15	1.41±.12	12.05±.74	.52±.04	9.20±1.19
	9	S 22.5	1.41±.18	12.94±1.05	.50±.04	11.72±1.77
	9	S 30	1.47±.12	12.99±.43	.48±.03	10.03±1.31
	6	SM 3.75	1.31±.11	13.34±.71	.51±.05	10.50±0.78
	9	SM 7.5	1.48±.11	13.06±.72	.50±.02	9.51±0.73
	9	SM 15	1.22±.09	13.04±.95	.56±.07	11.64±1.11
	9	SM 22.5	1.54±.11	13.09±.91	.43±.04	7.75±0.84
	9	SM 30	1.50±.17	12.69±1.45	.37±.03	8.73±0.83
	12	Stock	1.44±.09	11.49±1.03	.38±.03	8.40±0.65

Table 23. Results of Duncan's Test for the effect of varying levels of protein on DNA[a] - Experiment 3

% protein Series		7.5	15.0	22.5	30.0
S	DNA/g	1.25	1.41	1.41	1.47
	P< .01	----------			
	P< .05	_____			
SM	DNA/g	1.48	1.22	1.54	1.50
	P< .01	----------			
	P< .05	_____			

[a]Any means not underscored by the same line are significantly different.

Table 24. Results of Duncan's Test for the effect of methionine on DNA[a] - Experiment 3

% protein Group	7.5		15.0		22.5		30.0	
	S	SM	S	SM	S	SM	S	SM
DNA/g	1.25	1.48	1.41	1.22	1.41	1.54	1.47	1.50
P< .01	----------		----------		----------		----------	
P< .05	_____		_____		_____		_____	

[a]Any two means not underscored by the same line are significantly different.

Table 25. F values for regression equations of hepatic RNA - Experiment 3

Source of variation	d.f.	$\frac{RNA}{g}$	$\frac{RNA}{N}$	$\frac{RNA}{DNA}$
Regression	24	4.13**	2.37*	6.38**
Treatments	7	0.47	3.41**	3.10**
Methionine (M)	1	.62	-1.95	-1.11
Protein-linear (A)	1	.34	-3.25**	-.60
Protein-quadratic (B)	1	-.04	-.69	-.56
Protein-cubic (C)	1	-.44	.72	.44
Interaction MA	1	.71	.71	1.14
Interaction MB	1	.26	1.39	.16
Interaction MC	1	-.25	-1.10	-2.61
Litter	17	5.66**	1.95	7.38**
Error	47			

*Significant at 5% level.

**Significant at 1% level.

Table 26. Results of Duncan's Test for the effect of varying levels of protein on RNA[a] - Experiment 3

		S 7.5	S 15.0	S 22.5	S 30.0
RNA/g		12.3	12.1	12.9	13.0
	P< 0.01				
	P< 0.05				
		SM 7.5	SM 15.0	SM 22.5	SM 30.0
		13.1	13.0	13.1	12.7
	P< 0.01				
	P< 0.05				
		S 30	S 22.5	S 15	S 7.5
RNA/N		.48	.50	.52	.59
	P< 0.01				
	P< 0.05				
		SM 15	SM 7.5	SM 22.5	SM 30
		.56	.50	.43	.36
	P< 0.01				
	P< 0.05				
RNA/DNA		S 15	S 7.5	S 30	S 22.5
		9.20	10.2	10.0	11.7
	P< 0.01				
	P< 0.05				
		SM 15	SM 7.5	SM 30	SM 22.5
		11.6	9.51	8.7	7.8
	P< 0.01				
	P< 0.05				

[a]Any means not underscored by the same line are significantly different.

Table 27. Results of Duncan's Test for the effect of methionine on RNA[a] - Experiment 3

% protein	7.5		15.0		22.5		30.0	
Group	S	SM	S	SM	S	SM	S	SM
RNA/g	12.29	13.06	12.05	13.04	12.94	13.09	12.99	12.69
P< .01	----------		----------		----------		----------	
P< .05	----------		----------		----------		----------	
RNA/N	.59	.50	.52	.56	.50	.43	.48	.36
P< .01	----------		----------		----------		----------	
P< .05	----------		----------		----------		----------	
RNA/DNA	10.24	9.51	9.20	11.64	11.72	7.75	10.03	8.73
P< .01	----------		----------		-----	----	----------	
P< .05	----------		-----	-----	-----	----	----------	

[a]Any two means not underscored by the same line are significantly different.

For example, the difference in RNA/DNA ratios between groups SM 15 and SM 22.5 was probably a result of the previously mentioned variations in DNA values.

SDH

Activity of hepatic SDH was highly correlated with the quantity of unsupplemented protein (Experiment 3) whether based on unit tissue weight, DNA, or nitrogen (Table 28). In all instances, increases in protein concentrations in the diet from 7.5 to 30% produced an almost 3 fold increase in the activity of the enzyme (Tables 29, 30). For instance, values per g liver were 19.8 and 61.5 mg for groups S 7.5 and S 30 respectively.

Figure 7. Mean mg RNA (ribonucleic acid) per g liver in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiment 3.

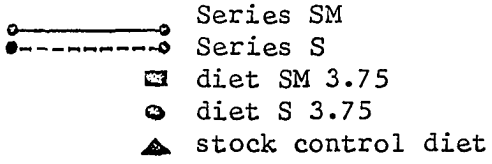


Figure 8. Mean hepatic RNA per mg nitrogen in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiment 3.

Figure 9. Mean hepatic RNA per mg DNA in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiment 3.

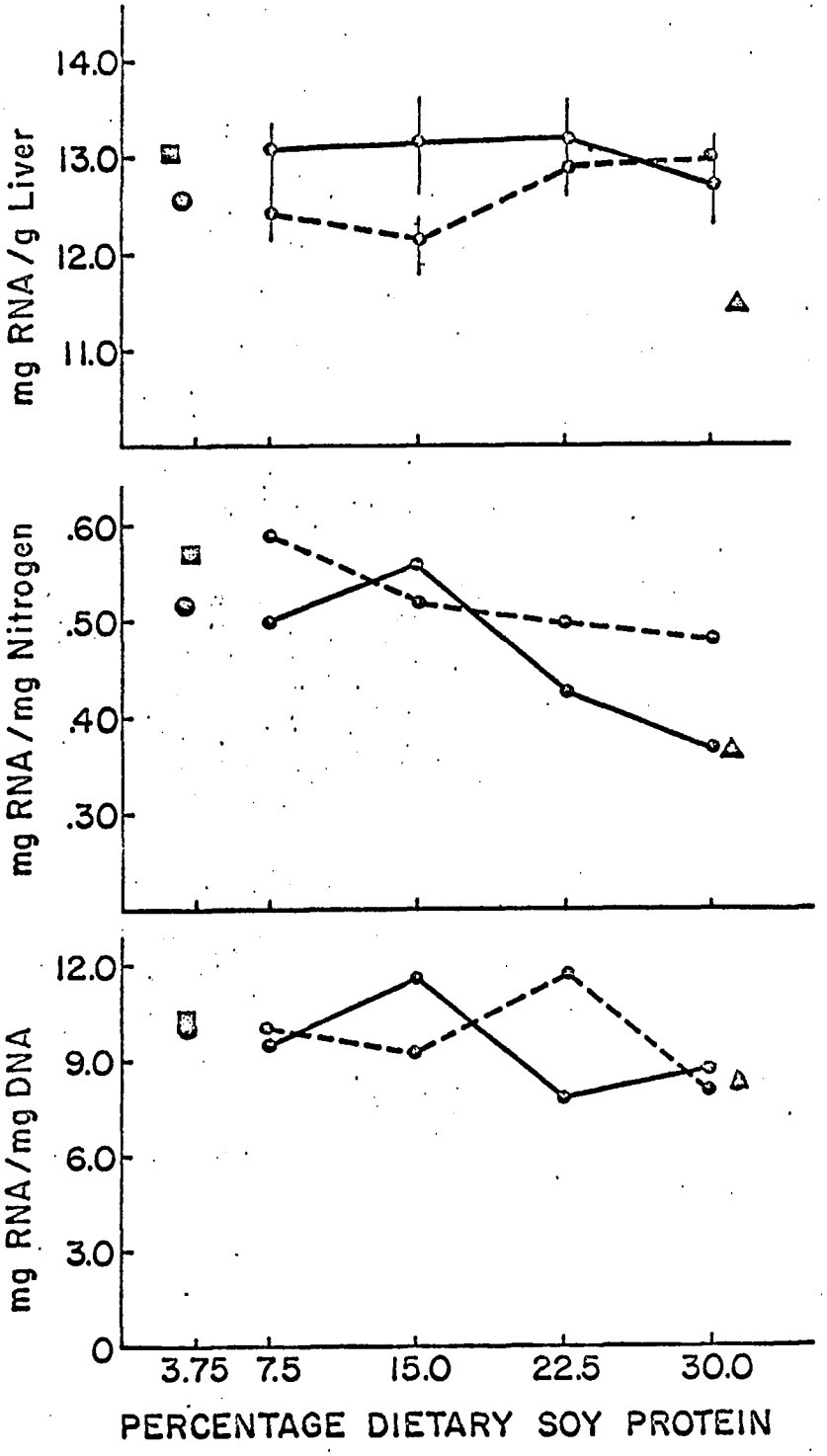

14.0
13.0
12.0
11.0
mg RNA/g Liver
.60
.50
.40
.30
mg RNA/mg Nitrogen
12.0
9.0
6.0
3.0
0
mg RNA/mg DNA
3.75
7.5
15.0
22.5
30.0
PERCENTAGE DIETARY SOY PROTEIN

Table 28. F values for regression equations of SDH activity - Experiment 3

Source of variation	d.f.	SDH / g	SDH / N	SDH / DNA
Regression	24	8.44**	4.78**	5.94**
Treatments	7	14.28**	6.10**	5.89**
Methionine (M)	1	3.46**	1.50	1.25
Protein-linear (A)	1	5.29**	2.51*	3.15**
Protein-quadratic (B)	1	-.33	-1.30	-.54
Protein-cubic (C)	1	1.02	1.78	1.49
Interaction MA	1	2.95*	3.68**	2.90*
Interaction MB	1	-.70	-.34	-.68
Interaction MC	1	.86	.38	-1.27
Litter	17	5.40**	3.92**	5.27**
Error	47			
Total	71			

*Significant at 5% level.

**Significant at 1% level.

Table 29. Mean hepatic SDH (mg formazan reduced/30 minutes) in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiment 3

Diet	no. rats	SDH / g liver	SDH / N	SDH / DNA
S 3.75	3	17.3[a]	.72[a]	11.6[a]
S 7.5	9	19.8±4.5[b]	.87±.19	17.1±4.4
S 15	9	42.1±6.6	1.84±.30	32.4±5.2
S 22.5	9	46.3±3.4	1.82±.18	42.2±6.0
S 30	9	61.5±4.3	2.28±.21	48.2±7.7
SM 3.75	3	34.0[a]	1.28[a]	32.0[a]
SM 7.5	9	49.6±6.2	1.93±.25	37.0±5.5
SM 15	9	52.4±6.0	2.19±.27	46.0±6.2
SM 22.5	9	55.3±4.6	1.82±.15	32.6±3.2
SM 30	9	60.8±2.4	1.78±.10	43.1±3.3
Stock	12	55.8±3.9	1.79± .10	36.8±3.3

[a]Not sufficient number of observations to calculate standard error.

[b]Standard error of mean.

Table 30. Results of Duncan's Test for the effect of varying levels of protein on SDH[a] - Experiment 3

% protein		7.5	15.0	22.5	30.0
S	SDH/g	19.8	42.1	46.3	61.5
	P< .01	-----	----------		-----
	P< .05	____	_____		____
SM	SDH/g	49.6	52.4	55.3	60.8
	P< .01	----------			
	P< .05	_____	_____		
S	SDH/N	.87	1.84	1.82	2.28
	P< .01	------	----------		
	P< .05	_____	_____		
SM	SDH/N	1.93	2.19	1.82	1.78
	P< .01	----------			
	P< .05	_____	_____		
S	SDH/DNA	1.71	3.24	4.22	4.82
	P< .01	------	----------	----------	
	P< .05	____	_____	_____	
SM	SDH/DNA	SM(22.5)	SM(7.5)	SM(30.0)	SM(15.0)
		3.23	3.69	4.31	4.66
	P< .01	----------			
	P< .05	_____	_____	_____	

[a]Any means not underscored by the same line are significantly different.

However, the progress in enzyme activity with increasing increments of protein concentration was not uniform. The most pronounced change in enzyme activity occurred with the rise in protein intake from 7.5 to 15%. Further reduction in SDH activity with a drop in the protein level to 3.75 could not be verified statistically.

The pattern of SDH response to variations in soy protein levels was significantly altered by the inclusion of methionine ($P < 0.01$) (Tables 28, 31; Figure 10). The rate of change in SDH concentration did not parallel the quantitative variations in protein content in SM diets since near maximum activity was seen when 15% supplemented protein was fed. In contrast to the unsupplemented series, the concentration in group SM 7.5 was only 20% below the highest value observed. No differences were seen among groups fed SM diets when activity was expressed per mg nitrogen or per mg DNA with the exception of group SM 22.5 which had less SDH activity per mg DNA than groups SM 15.

The inclusion of methionine increased the activity of SDH ($P < 0.01$) (Table 28). However, when means of isonitrogenous diets were compared, only groups receiving 7.5% protein showed a statistically significant response to methionine supplementation (Table 31).

In general, results of Experiment 3 confirmed those obtained in the second study, since mean values of SDH activity per g liver for comparable groups of Experiment 2 and 3 were in fairly good agreement (Table 32, Figures 13, 14). Absolute values for groups SM 15 of the two studies showed the greatest divergence, but these were comparable to their respective stock control values. Enzyme activity for Experiment 1 was low relative

Table 31. Results of Duncan's Test on effect of methionine supplementation on SDH activity[a] - Experiment 3

% protein	7.5		15.0		22.5		30.0	
Group	S	SM	S	SM	S	SM	S	SM
SDH/g	19.8	49.6	42.1	52.4	46.3	55.3	61.5	60.8
P< .01	----	----	----------		----------		----------	
P< .05	____	____	_____		_____		_____	
SDH/N	.87	1.93	1.84	2.19	1.82	1.82	2.28	1.78
P< .01	----	-----	----------		----------		----------	
P< .05	____	_____	_____		_____		_____	
SDH/DNA	1.71	3.69	3.24	4.66	4.21	3.23	4.82	4.31
P< .01	----	----	----------		----------		----------	
P< .05	____	____	____	____	_____		_____	

[a]Any two means not underlined by the same line are significantly different.

to other series. SDH activity had been determined by procedures described by Nachlas, et al. (1961) in Experiment 1, but measured according to the methods of Muramatsu and Ashida (1961) in Experiments 2 and 3. One might be tempted to suggest that part of the discrepancy in absolute values between series was attributable to variations in methodology, but values for rats fed stock rations were similar in Experiments 1 and 2, indicating that methodology was probably of minor importance only.

In Experiment 1, the quantity of protein in the diet influenced SDH concentration more than the type or supplementation of proteins studied. Mean values for rats maintained on 7.5% protein were approximately two-

Figure 10. Mean SDH activity per g liver in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiment 3.

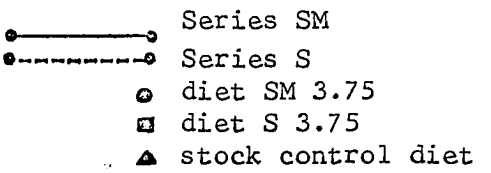


Figure 11. Mean hepatic SDH per mg nitrogen in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiment 3.

Figure 12. Mean hepatic SDH per mg DNA in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiment 3.

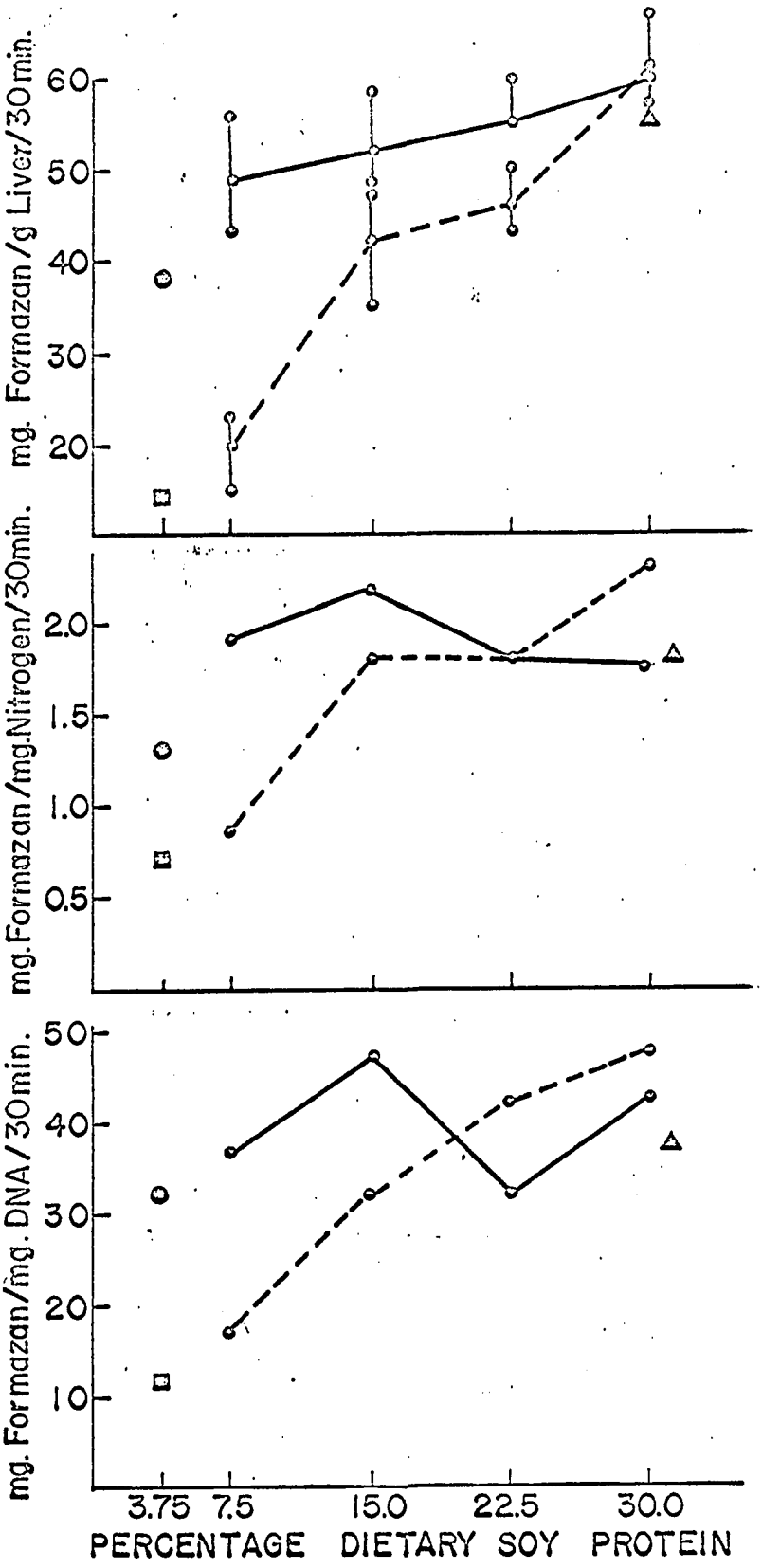

mg. Formazan /g Liver/30 min.
60
50
40
30
20
mg.Formazan/mg.Nitrogen/30min.
2.0
1.5
1.0
0.5
mg. Formazan/mg. DNA/30 min.
50
40
30
20
10
3.75
7.5
15.0
22.5
30.0
PERCENTAGE DIETARY SOY PROTEIN

Figure 13. Mean SDH activity per g liver in young female rats fed varying quantities of soy protein without methionine for 28 days - Experiments 2 and 3.

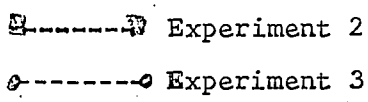
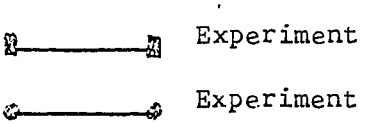
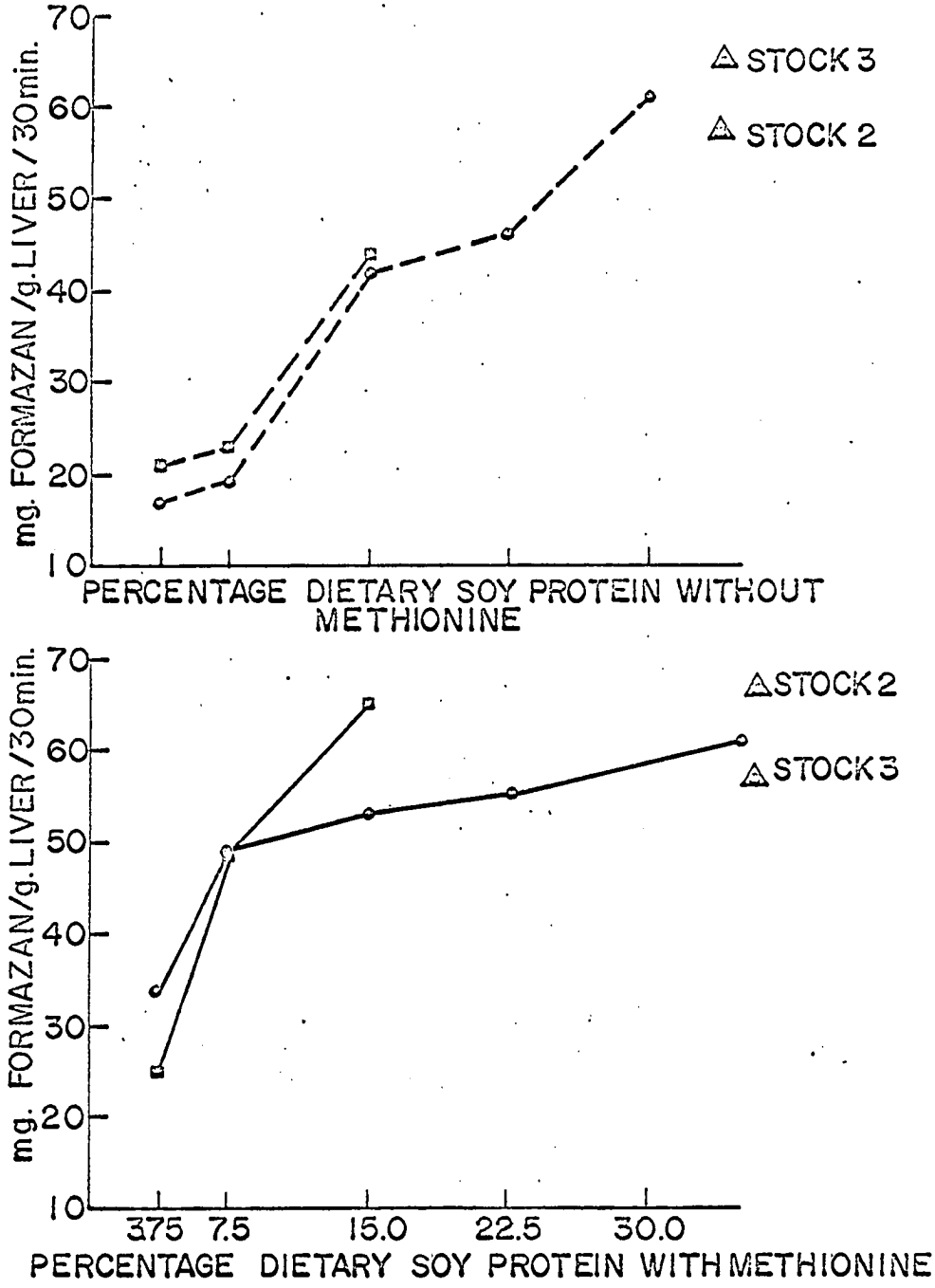


Figure 14. Mean SDH activity per g liver in young female rats fed varying quantities of soy protein with methionine for 28 days - Experiments 2 and 3.




70
60
50
40
30
20
10
mg. FORMAZAN /g. LIVER / 30 min.
STOCK 3
STOCK 2
PERCENTAGE DIETARY SOY PROTEIN WITHOUT METHIONINE
70
60
50
40
30
20
10
mg. FORMAZAN/g. LIVER/30min.
STOCK 2
STOCK 3
3.75
7.5
15.0
22.5
30.0
PERCENTAGE DIETARY SOY PROTEIN WITH METHIONINE

thirds of those on 15% protein. No apparent differences could be associated with the feeding of lactalbumin or soy protein (Table 32). Lowest and highest concentrations of SDH were observed with the protein free and the stock diet respectively.

Table 32. Mean SDH, GPT, and RNAse activity per g liver in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiments 1, 2, 3

Expt. no.	Diet	SDH mg [a]	GPT units X 10^{-3}	RNAse mg
1	PF	9.8	19.9	5.58
	L 7.5	15.2	35.8	1.06
	L 15	25.8	37.4	.91
	LM 7.5	12.2	39.6	1.01
	LM 15	22.3	36.6	.77
	SM 7.5	13.2	42.7	1.05
	SM 15	21.0	39.0	1.02
	S4M 7.5	13.3	39.1	1.41
	S4M 15	21.0	32.0	1.03
	Stock control	47.4	46.4	.91
2	S 3.75	21.1	22.1	1.3
	S 7.5	22.5	25.0	1.5
	S 15	44.0	28.4	1.4
	SM 3.75	24.6	28.4	1.2
	SM 7.5	49.6	36.7	1.7
	SM 15	65.5	41.0	1.7
	Stock control	65.2	46.0	1.6
3	S 3.75	17.3	44.7	.90
	S 7.5	19.8	41.7	1.03
	S 15	42.1	52.6	.81
	S 22.5	46.3	53.7	.78
	S 30	61.5	67.3	.82
	SM 3.75	34.0	46.8	1.05
	SM 7.5	49.6	49.2	.77
	SM 15	52.4	52.4	.75
	SM 22.5	55.3	67.2	.72
	SM 30	60.8	77.1	.75
	Stock control	55.8	66.0	.72

[a] mg formazan reduced per 30 minutes.

GPT

Stepwise increments of unsupplemented soy protein from 7.5 to 30% produced increases in concentration of GPT ($P < 0.01$) (Table 33). Significant differences in mean GPT concentrations existed between the lowest and highest protein levels of the series. Values were 41.7 and 67.3 x 10^3 units per g liver respectively (Table 34, Figure 15). Similarly activity of GPT relative to DNA was influenced by increases in protein intake ($p < 0.05$) (Table 34, Figure 17). Since the GPT/N ratio was not altered by nutritional variations, it appears that GPT activity and nitrogen concentrations were affected to the same extent (Table 33). Mean ratios ranged from 1.96 to 2.52 x 10^3 units (Table 34, Figure 16).

In animals fed supplemented protein diets, mean value of GPT per g liver was greater for group SM 30 than for SM 7.5 and SM 15 (Table 35, Figure 15). No change in mean GPT/N or GPT/DNA was seen in animals maintained on different levels of supplemented soy protein (Table 35, Figures 16, 17).

The inclusion of methionine into diets did not affect GPT activity whether expressed relative to wet weight, nitrogen or DNA (Tables 33, 36; Figures 15, 16, 17).

The magnitude of GPT response may have been modified by other factors in addition to dietary variations. Groups in Experiment 3 had higher mean values than those of comparable groups in Experiment 2, even in the stock control groups (Table 32, Figures 18, 19). However, it appears that in all three experiments, groups SM 15 had GPT values which were within the range of respective stock control values.

Table 33. F values for regression equation for hepatic GPT activity $X 10^{-3}$ - Experiment 3

Source of variation	d.f.	GPT / g	GPT / N	GPT / DNA
Regression	24	2.57*	1.87*	2.97**
Treatments	7	3.75**	8.43**	1.68
Methionine (M)	1	1.56	-.35	-.18
Protein-linear (A)	1	4.01**	1.47	2.14*
Protein-quadratic (B)	1	.48	-.15	-.01
Protein-cubic (C)	1	.13	.97	.25
Interaction MA	1	-.48	.18	.54
Interaction MB	1	-.21	.36	-.55
Interaction MC	1	.88	.53	-1.43
Litter	17	2.12*	2.35*	3.48**
Error	47			

*Significant at 5% level.

**Significant at 1% level.

Table 34. Mean glutamic pyruvic transaminase activity X 10^{-3} in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiment 3

Diet	No. rats	GPT / g liver	GPT / N	GPT / DNA
S 3.75	4	44.7±6.0	1.79±.21	35.1±4.5
S 7.5	9	41.7±5.0	1.96±.19	34.8±4.6
S 7.5	9	52.6±3.2	2.33±.18	38.6±2.8
S 22.5	9	53.7±8.3	2.08±.30	53.5±15.0
S 30	9	67.3±9.5	2.52±.42	51.3±9.0
SM 3.75	4	46.8±4.7	1.83±.24	40.6±6.3
SM 7.5	9	49.2±3.9	1.91±.18	37.2±6.1[a]
SM 15	9	52.4±6.7	2.20±.32	46.3±6.7
SM 22.5	9	67.2±7.2	2.20±.22	38.0±3.0
SM 30	9	77.1±8.9	2.29±.31	52.8±5.9
Stock	12	66.0±6.0	2.27±.49	51.2±5.1

[a]Standard error of mean.

Figure 15. Mean units GPT per g liver in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiment 3.

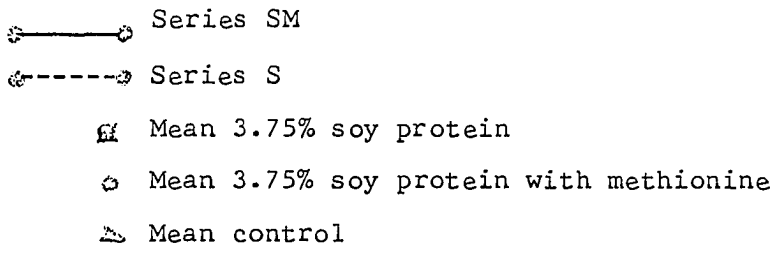


Figure 16. Mean hepatic GPT per mg nitrogen in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiment 3.

Figure 17. Mean hepatic GPT per mg DNA in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiment 3.

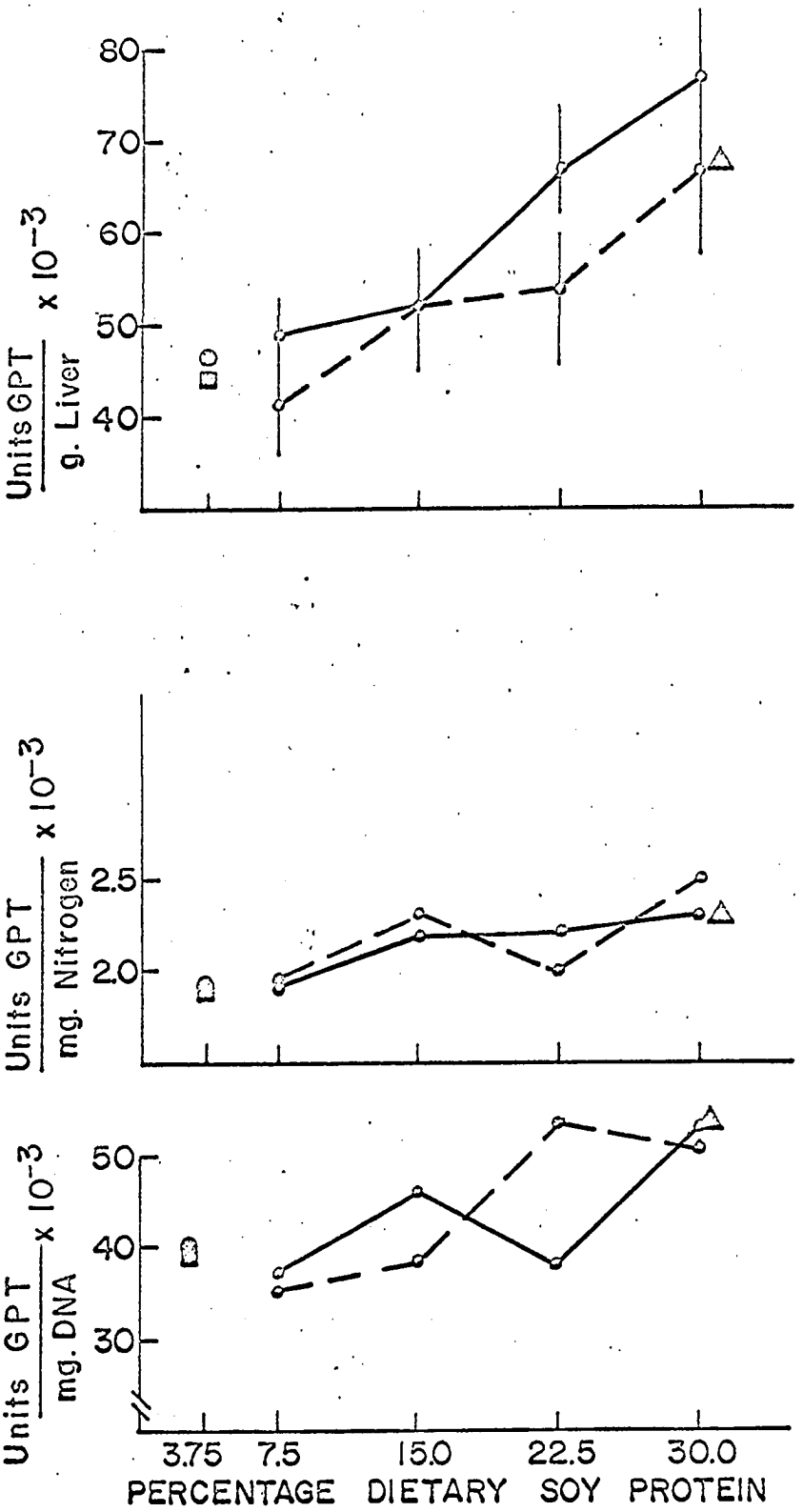

80
70
60
50
40
Units GPT / g. Liver x 10⁻³
2.5
2.0
Units GPT / mg. Nitrogen x 10⁻³
50
40
30
Units GPT / mg. DNA x 10⁻³
3.75
7.5
15.0
22.5
30.0
PERCENTAGE DIETARY SOY PROTEIN

Table 35. Results of Duncan's Test for the effect of varying levels of protein on GPT[a] activity X 10^{-3} - Experiment 3

	% Protein	7.5	15.0	22.5	30.0
S	GPT/g	41.7	52.6	53.7	67.3
	P< .01	----------			
	P< .05	_____	_____		
SM	GPT/g	49.2	52.4	67.2	77.1
	P< .01	----------			
	P< .05	_____	_____	_____	
S	GPT/N	1.96	2.33	2.08	2.52
	P< .01	----------			
	P< .05	_____	_____		
SM	GPT/N	1.91	2.20	2.20	2.29
	P< .01	----------			
	P< .05	_____	_____		
S	GPT/DNA	34.8	39.6	53.5	51.3
	P< .01	----------			
	P< .05	_____	_____		
SM	GPT/DNA	37.2	46.3	38.0	52.8
	P< .01	----------			
	P< .05	_____	_____		

[a]Any two means not underscored by the same line are significantly different. Comparisons may be between groups of means.

The quantity and quality of dietary proteins investigated in Experiment 1 and 2 did not affect the concentration of GPT over the short range of protein intakes studied (Table 32, Figures 18, 19). Means of animals maintained on 7.5% proteins appeared similar to those receiving 15% protein. In the first experiment, values associated with soy diets did not appear different from those seen with lactalbumin diets.

Table 36. Results of Duncan's Test on the effect of methionine supplementation on GPT activity[a] X 10^{-3} - Experiment 3

% protein	7.5		15.0		22.5		30.0	
Group	S	SM	S	SM	S	SM	S	SM
GPT/g	41.7	49.2	52.6	52.4	53.7	67.2	67.3	77.1
P< .01	----------		----------		----------		----------	
P< .05	_____		_____		_____		_____	
GPT/N	1.96	1.91	2.20	2.33	2.08	2.20	2.29	2.52
P< .01	----------		----------		----------		----------	
P< .05	_____		_____		_____		_____	
GPT/DNA	34.8	37.2	39.6	46.3	53.5	38.0	51.6	52.8
P< .01	----------		----------		----------		----------	
P< .05	_____		_____		_____		_____	

[a]Any two means not underscored by the same line are significantly different.

RNAse

A narrow range of RNAse concentrations existed over the entire range of dietary variables investigated and values did not reflect increments of protein (Tables 37, 38; Figure 20). Since under similar nutritional conditions, the DNA concentration had not varied, changes in RNAse/DNA ratios were also not observed. However, RNAse activity was negatively related to nitrogen concentration because incorporation of graded quantities of soy protein into diets had produced an elevation in hepatic nitrogen. This was apparent when regression equations were calculated for RNAse/N ratios for both series of soy protein diets, with or without added

Figure 18. Mean GPT activity per g liver in young female rats fed varying quantities of soy protein without methionine for 28 days - Experiments 2 and 3.

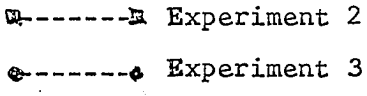
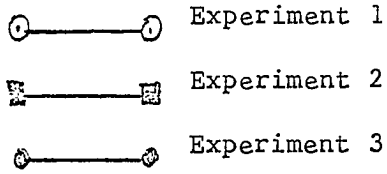
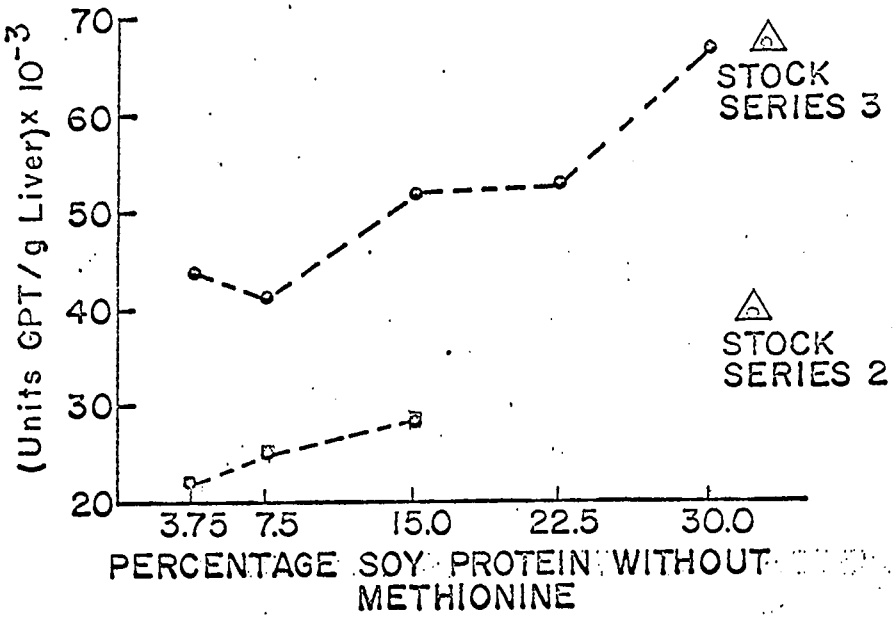


Figure 19. Mean GPT activity per g liver in young female rats fed varying quantities of soy protein with methionine for 28 days - Experiments 1, 2, and 3.




(Units GPT/g Liver)x 10-3
70
60
50
40
30
20
STOCK SERIES 3
STOCK SERIES 2
3.75 7.5 15.0 22.5 30.0
PERCENTAGE SOY PROTEIN WITHOUT METHIONINE

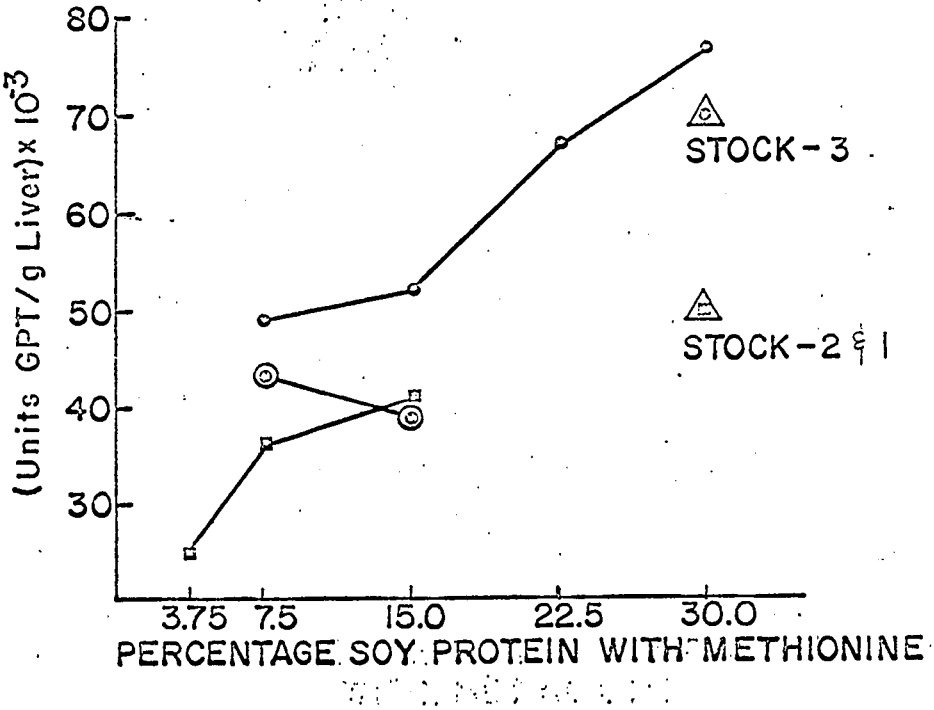

(Units GPT/g Liver)x 10-3
80
70
60
50
40
30
STOCK-3
STOCK-2 & 1
3.75 7.5 15.0 22.5 30.0
PERCENTAGE SOY PROTEIN WITH METHIONINE

Table 37. F values for regression equations of hepatic RNAse activity - Experiment 3

Source of variation	d.f.	RNAse / g	RNAse / N	RNAse / DNA
Regression	24	1.83	2.71**	2.12·
Treatments	7	1.12	9.78**	20.44**
Methionine (M)	1	-1.36	-2.84**	-1.86
Protein-linear (A)	1	-1.00	-2.98**	-1.26
Protein-quadratic (B)	1	.97	.79	.74
Protein-cubic (C)	1	-.05	.25	.21
Interaction MA	11	-.78	-1.07	-.21
Interaction MB	1	.65	1.33	.35
Interaction MC	11	-.27	-.75	-1.71
Litter	17	2.20	4.45**	2.30*
Error	47			

*Significant at 5% level.

**Significant at 1% level.

Table 38. Mean hepatic ribonuclease activity in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiment 3

Diet	no. rats	RNAse / g liver	RNAse / N	RNAse / DNA
S 3.75	3	.90[a]	.040[a]	.60[a]
S 7.5	9	1.03±.12[b]	.051±.007	.85±.10[b]
S 15	9	.81±.15	.035±.007	.60±.13
S 22.5	9	.78±.10	.031±.004	.71±.15
S 30	9	.82±.11	.030±.004	.63±.12
SM 3.75	3	1.05[a]	.040[a]	.90[a]
SM 7.5	9	.77±.20	.029±.007	.59±.16
SM 15	9	.75±.08	.031±.004	.66±.07
SM 22.5	9	.72±.06	.023±.002	.41±.03
SM 30	9	.75±.08	.022±.002	.54±.08
Stock	12	.72	.022±	.52±

[a]Insufficient number of observations to calculate standard error.

[b]Standard error of mean.

Figure 20. Mean RNAse activity per g liver in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiment 3.

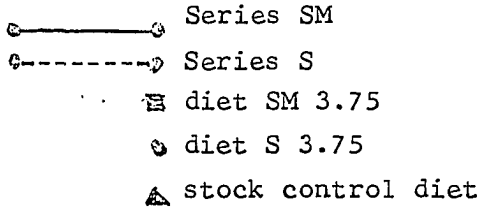
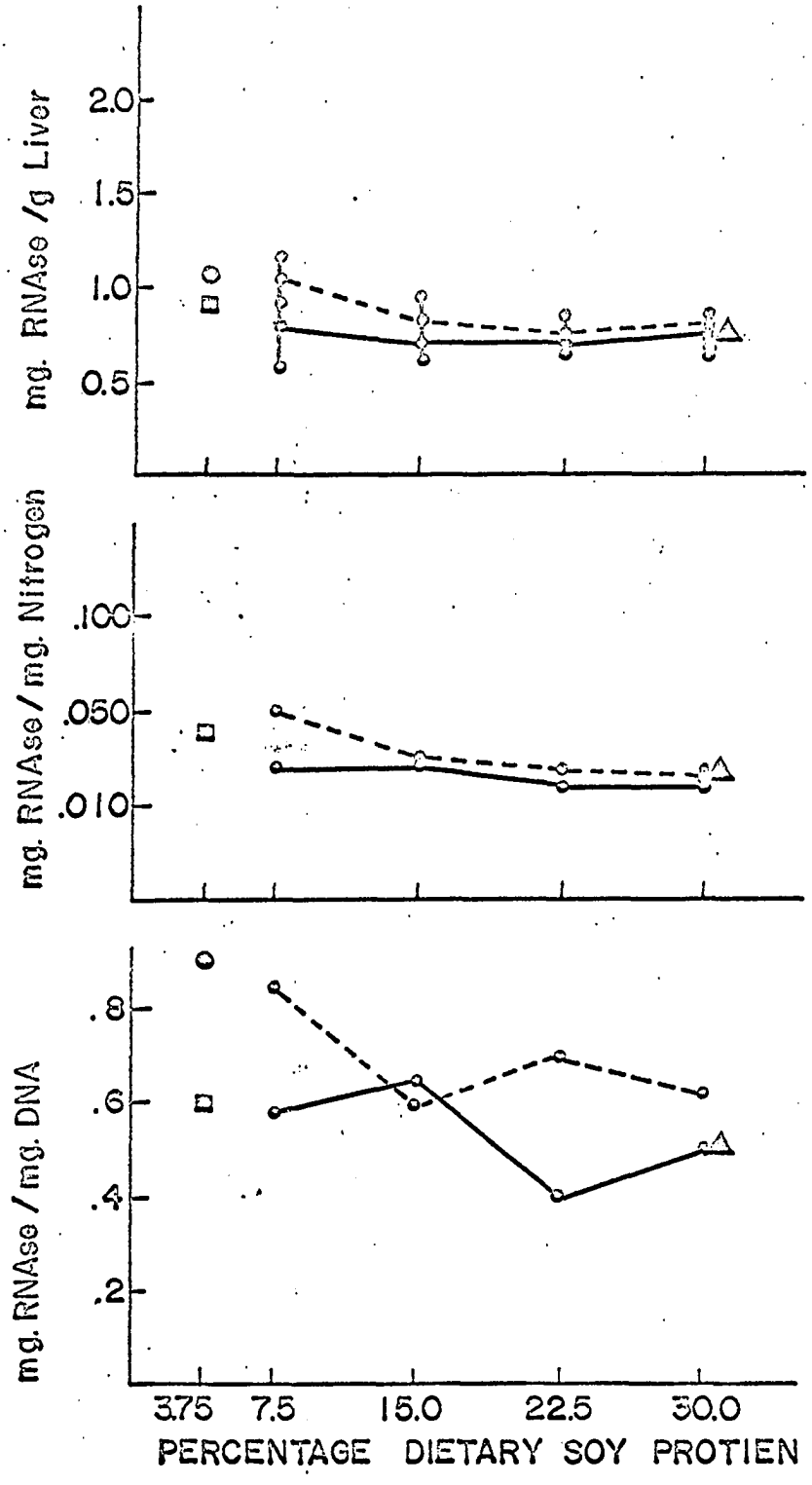


Figure 21. Mean hepatic RNAse per mg nitrogen in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiment 3.

Figure 22. Mean hepatic RNAse per mg DNA in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiment 3.


mg. RNAse /g Liver
2.0
1.5
1.0
0.5
mg. RNAse / mg. Nitrogen
.100
.050
.010
mg. RNAse / mg. DNA
.8
.6
.4
.2
3.75
7.5
15.0
22.5
30.0
PERCENTAGE DIETARY SOY PROTIEN

methionine ($P < 0.01$). Ratios for RNAse/N varied between 0.051 and 0.030 for unsupplemented groups and 0.031 and 0.022 for supplemented groups.

RNAse activity, on the basis of wet weight, or DNA, was refractory to the addition of methionine as it was to the elevation of the protein intake (Tables 39, 37 ; Figures 22). However, regression based on values from individual animals demonstrated that the RNAse/N ratio was significantly less in groups receiving the supplemented protein ($P < 0.01$). Comparison of mean RNAse/N ratios of S and SM groups receiving isonitrogenous diets showed that a difference in values existed only in groups receiving 7.5% protein (Figure 21).

As in Experiment 3, the influence of quantity or quality of protein was not statistically apparent on RNAse activity per g tissue in the other experiments (Table 32). There were no differences observed among animals receiving stock, 7.5, or 15% protein diets. Also, rats fed lactalbumin or soy protein, regardless of supplementation, had similar concentrations of RNAse.

The effect of an acute protein deficiency on RNAse activity was evident in Experiment 1, where the concentration of RNAse associated with the feeding of the protein-free diet was 5.6 mg per g liver or 5 times greater than that in animals fed other diets (Table 32). When RNAse was expressed on the basis of whole liver, the difference between protein-free and other diets diminished, but the mean value for protein deficient rats was still approximately 175% greater than that for other groups.

Table 39. Results of Duncan's Test for the effect of varying levels of protein on RNAse[a] activity - Experiment 3

	% protein	7.5	15.0	22.5	30.0
S	RNAse/g	1.03	.81	.78	.82
	P< .01	----------	----------	----------	----------
	P< .05	----------	----------	----------	----------
SM	RNAse/g	.77	.75	.72	.75
	P< .01	----------	----------	----------	----------
	P< .05	----------	----------	----------	----------
S	RNAse/N	.051	.035	.031	.030
	P< .01	----	----------	----------	----------
	P< .05	----	----------	----------	----------
SM	RNAse/N	.029	.031	.023	.022
	P< .01	----------	----------	----------	----------
	P< .05	----------	----------	----------	----------
S	RNAse/DNA	.85	.60	.71	.64
	P< .01	----------	----------	----------	----------
	P< .05	----------	----------	----------	----------
SM	RNAse/DNA	.59	.66	.41	.54
	P< .01	----------	----------	----------	----------
	P< .05	----------	----------	----------	----------

[a]Any two means not underscored by the same line are significantly different. Comparison may be between groups of means.

Table 40. Results of Duncan's Test on the effect on methionine on RNAse activity[a] - Experiment 3

% protein	7.5		15.0		22.5		30.0	
Group	S	SM	S	SM	S	SM	S	SM
RNAse/g	1.03	.77	.81	.75	.78	.72	.82	.75
P< .01	----------		----------		---------		----------	
P< .05	_____		_____		_____		_____	
RNAse/N	.051	.029	.035	.031	.031	.023	.030	.022
P< .01	----	----	----------		---------		----------	
P< .05	____	____	_____		_____		_____	
RNAse/DNA	.85	.59	.60	.66	.71	.41	.64	.54
P< .01	----------		---------		---------		---------	
P< .05	_____		_____		_____		_____	

[a]Any two means not underscored by the same line are significantly different.

FAO-8

FAO-8 activity was determined in Experiments 2 and 3. Differences in the magnitude of activity of FAO-8 of comparable groups in the 2 experiments were evident (Tables 41, 42), obliterating a distinct pattern of response of the enzyme to dietary variations. However, there was a trend toward increased activity of the enzyme when protein intake was raised from 7.5 to 15%.

In Experiment 3, stepwise increments of unsupplemented soy protein led to a rise in hepatic FAO-8 activity ($P < 0.01$) (Tables 41, 42; Figure

Table 41. FAO-8 (uLO_2 uptake per g liver per minute with caprylic acid as substrate) in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiments 2, 3

Exp.	Groups	No. rats	FAO-8 / g liver	FAO-8 / N	FAO-8 / DNA
2	S 3.75	10	24.6±3.3[a]		
	S 7.5	10	22.4±3.0		
	S 15.0	10	28.2±3.1		
	SM 3.75	10	25.6±4.3		
	SM 7.5	10	31.6±3.3		
	SM 15.0	10	39.4±3.6		
	Stock	10	57.0±4.1		
3	S 3.75	6	21.4±3.0	.97±.17	18.0±4.2
	S 7.5	9	20.3±3.6	.98±.18	16.7±2.9
	S 15.0	9	36.7±3.3	1.58±.11	28.2±4.0
	S 22.5	9	42.7±3.6	1.65±.13	37.0±3.6
	S 30.0	9	51.9±5.4	1.89±.17	38.0±4.0
	SM 3.75	6	33.6±1.9	1.30±.20	28.6±3.9
	SM 7.5	9	38.6±8.0	1.46±.31	28.6±5.9
	SM 15.0	9	39.7±4.5	1.79±.41	35.8±4.9
	SM 22.5	9	55.4±8.1	1.85±.30	33.2±5.4
	SM 30	9	56.6±1.9	1.70±.13	41.2±4.2
	Stock	12	52.6±4.9	1.69±.18	37.6±3.4

[a]Standard error of mean.

23). Mean FAO-8 concentration of group S 30 exceeded that of group S 7.5 by 250% (Table 43). Values associated with diets S 15 and S 22.5 were intermediate. Mean enzyme activity of group S 30 was comparable to that of stock controls, indicating that optimal concentration of enzyme activity may have been reached.

A similar pattern of response of the FAO-8 system was apparent when activity of unsupplemented groups was calculated relative to either nitrogen

Table 42. F values for regression equations for hepatic FAO-8 activity - Experiment 3

Source of variation	d.f.	FAO-8 / g	FAO-8 / N	FAO-8 / DNA
Regression	24	3.83**	1.98*	2.64**
Treatments	7	7.60**	2.65*	3.77**
Methionine (M)	1	2.60*	-1.03	1.50
Protein-linear (A)	1	5.14**	2.34*	3.83**
Protein-quadratic (B)	1	-.47	-1.25	-.77
Protein-cubic (C)	1	-.46	.50	.56
Interaction MA	1	.93	1.33	1.33
Interaction MB	1	-.49	.17	-.89
Interaction MC	1	1.28	.41	-.91
Litter	17	2.44*	2.02*	2.11*
Error	47			

*Significant at 5% level.

**Significant at 1% level.

or DNA, but the degree of significance varied from $P < 0.01$ for FAO-8/DNA to $P < 0.05$ for FAO-8/N (Tables 41, 43; Figures 24, 25).

The trend in behavior of the FAO-8 enzyme to the feeding of graded quantities of supplemented soy protein was similar to that described for the giving of unsupplemented soy protein. Enzyme activity expressed per unit hepatic tissue increased with elevating the protein intake (Table 43,

Table 43. Results of Duncan's Test for the effect of varying levels of protein on FAO-8 activity[a] - Experiment 3

	% protein	7.5	15.0	22.5	30.0
S	FAO-8/g	20.3	36.7	42.7	51.9
	P< .01	----------	----------	----------	
	P< .05	____	_____	_____	
SM	FAO-8/g	38.6	39.7	55.4	56.6
	P< .01	----------	----------	----------	
	P< .05	_____	_____	_____	
S	FAO-8/N	.987	1.58	1.65	1.89
	P< .01	----------	----------	----------	
	P< .05	_____	_____	_____	
SM	FAO-8/N	1.46	1.79	1.85	1.66
	P< .01	----------	----------	----------	
	P< .05	_____	_____	_____	
S	FAO-8/DNA	16.9	28.2	37.0	38.0
	P< .01	----------	----------	----------	
	P< .05	____	_____	_____	
SM	FAO-8/DNA	28.6	35.8	33.2	40.0
	P< .01	----------	----------	----------	
	P< .05	_____	_____	_____	

[a]Any two means not underscored by the same line are significantly different.

Figure 23. Mean FAO-8 activity (uL O_2 uptake) per g liver in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiment 3.

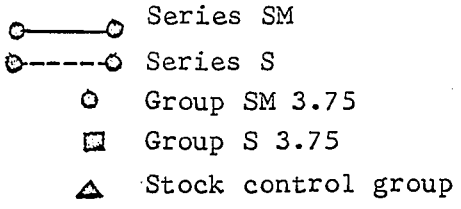


Figure 24. Mean hepatic FAO-8 per mg nitrogen in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiment 3.

Figure 25. Mean hepatic FAO-8 per mg DNA in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiment 3.

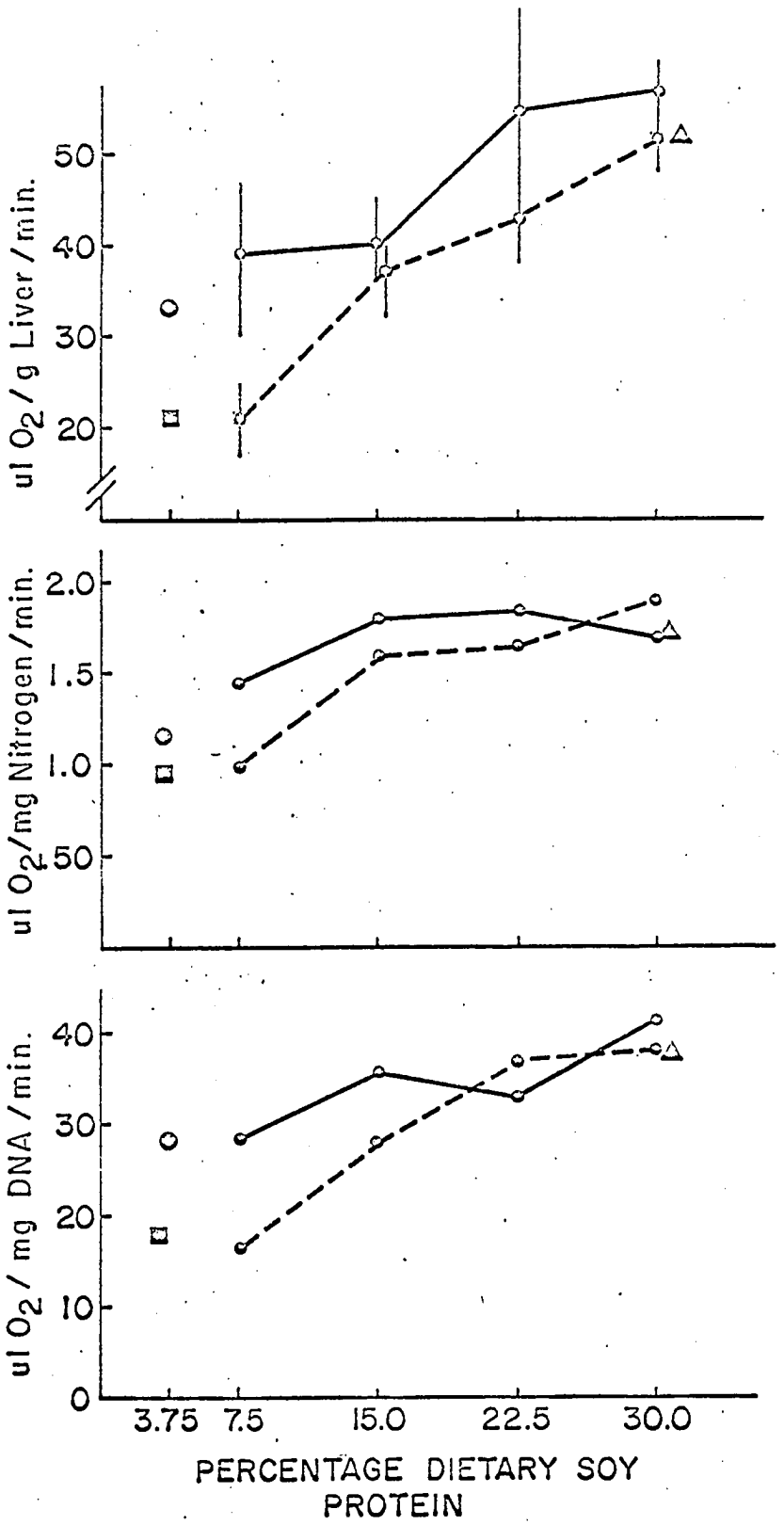

ul O2 / g Liver / min.
50
40
30
20
ul O2/mg Nitrogen / min.
2.0
1.5
1.0
.50
ul O2 / mg DNA / min.
40
30
20
10
0
3.75
7.5
15.0
22.5
30.0
PERCENTAGE DIETARY SOY PROTEIN

Figure 23). However, this relationship could not be demonstrated when FAO-8 activity was related to nitrogen or DNA (Table 42, Figures 24, 25).

It appears that near maximum FAO-8 activity was reached when the protein level in the diet was raised to 30% regardless of supplementation, since the FAO-8 value for stock controls was within the range of groups S 30 and SM 30. At an intake of 22.5% protein, methionine was apparently needed to produce maximum activity as the value for the unsupplemented group fell within the range of stock controls.

The addition of methionine was related to an increase in FAO-8 activity when expressed on the basis of wet liver ($P < 0.05$), but not when activity was related to DNA or nitrogen (Table 44). However, comparison of groups on isonitrogenous diets showed that the activity associated with diet SM 7.5 was significantly raised when activity was expressed on the basis of wet weight, or DNA.

FAO-16

FAO-16 activity was determined in Series 3. In instances where livers were small, the quantity of hepatic tissue available was inadequate for all analyses planned. For this reason, hepatic FAO-16 data were obtained from 24 rats only. Therefore, 24 rats were added to those in the original plan in order to provide a minimum of 6 rats per treatment group (Experiment 3-A). Standard errors for FAO-16 values were large. Because of these two factors, (Table 45) F-values for regression equations of FAO-16 data carry greater significance than mean values.

When the quantity of soy protein without added methionine was increased, the concentration of FAO-16 appeared to change in a parallel

Table 44. Results of Duncan's Test on the effect of methionine on FAO-8 activity[a] - Experiment 3

% protein	7.5		15.0		22.5		30.0	
Group	S	SM	S	SM	S	SM	S	SM
FAO-8/g	20.3	38.6	36.7	39.7	42.7	55.4	51.9	56.6
P< .01	----	----	----------		----------		----------	
P< .05	———	———	———————		———————		———————	
FAO-8/N	.98	1.46	1.58	1.79	1.65	1.85	1.89	1.66
P< .01	----------		----------		----------		----------	
P< .05	———————		———————		———————		———————	
FAO-8/DNA	16.9	28.6	28.2	35.8	37.0	33.2	38.0	40.0
P< .01	----------		----------		----------		----------	
P< .05	———	———	———————		———————		———————	

[a]Any two means not underscored by the same line are significantly different.

manner (P< 0.05) (Tables 45, 46; Figure 26), but differences in mean concentrations were not detected (Table 47). Under similar dietary conditions, FAO-16 activity increased when related to nitrogen (P< 0.05) (Tables 45, 47; Figure 27). FAO-16/DNA ratios also increased as the protein was elevated (p< 0.01) (Tables 45, 47; Figure 28). When FAO-16 was related to DNA, the mean ratio for groups S 22.5 was significantly larger than that for group S 7.5.

In animals fed graded amounts of supplemented soy protein, FAO-16 increased whether activity was expressed on the basis of wet weight,

Table 45. FAO-16 (uLO_2 uptake per g liver per minute with palmityl carnitine as substrate) activity in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiment 3-A

Groups	no. rats	FAO-16 / g	FAO-16 / N	FAO-16 / DNA
S 3.75	3	4.7±0.9[a]	.21±.06	3.8±0.8
S 7.5	6	3.7±0.8	.18±.03	2.5±0.5
S 15.0	6	4.5±1.4	.19±.06	3.5±1.1
S 22.5	6	7.3±2.4	.29±.09	8.2±2.5
S 30.0	6	7.5±1.7	.30±.07	5.5±1.3
SM 3.75	3	3.9±0.8	.14±.06	2.8±0.6
SM 7.5	6	6.0±1.4	.26±.06	5.2±1.2
SM 15.0	6	8.3±1.6	.30±.05	7.4±1.9
SM 22.5	6	8.7±1.5	.33±.07	6.4±1.1
SM 30.0	6	13.1±2.2	.42±.07	12.6±3.4
Stock	4	19.6±1.8	.73±.06	14.9±1.5

[a]Standard error of mean.

Table 46. F values for regression equations on hepatic FAO_{16} activity - Experiment 3-A

Source of variation	d.f.	FAO_{16} / g	FAO_{16} / N	FAO_{16} / DNA
Regression	18	3.30*	2.91**	2.36*
Treatments	7	4.15**	2.47*	2.65*
Methionine (M)	1	2.70*	1.87	2.25
Protein-linear (A)	1	3.38**	2.47*	3.00**
Protein -quadratic (B)	1	.28	.29	.07
Protein-cubic (C)	1	.11	-.27	-1.02
Interaction MA	1	-.71	-.14	-.63
Interaction MB	1	-.58	-.23	-1.44
Interaction MC	1	-1.00	-.51	-1.84
Litter	11	2.67*	3.27**	1.69
Error	29			

*Significant at 5% level.

**Significant at 1% level.

Table 47. Results of Duncan's Test for the effect of varying levels of protein on FAO-16[a] - Experiment 3-A

	% protein	7.5	15.0	22.5	30.0
S	FAO-16/g	3.7	4.5	7.3	7.5
	P< .01	----------	----------	----------	----------
	P< .05	_____	_____	_____	_____
SM	FAO-16/g	6.0	8.3	8.7	13.1
	P< .01	----------	----------	----------	----------
	P< .05	_____	_____	_____	_____
S	FAO-16/N	.18	.19	.29	.30
	P< .01	----------	----------	----------	----------
	P< .05	_____	_____	_____	_____
SM	FAO-16/N	.26	.30	.33	.42
	P< .01	----------	----------	----------	----------
	P< .05	_____	_____	_____	_____
SM	FAO-16/DNA	5.2	7.4	6.4	12.6
	P< .01	----------	----------	----------	----------
	P< .05	_____	_____	_____	_____
S	FAO-16/DNA	2.5	3.5	(30.0)[b] 5.5	(22.5) 8.2
	P< .01	----------	----------	----------	----------
	P< .05	_____	_____	_____	_____

[a]Any 2 means not underscored by the same line are significantly different.

[b]Higher activity obtained with diet S 22.5 than S 30.0.

Figure 26. Mean FAO-16 activity (uL O_2 uptake) per g liver in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiment 3-A.

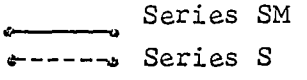
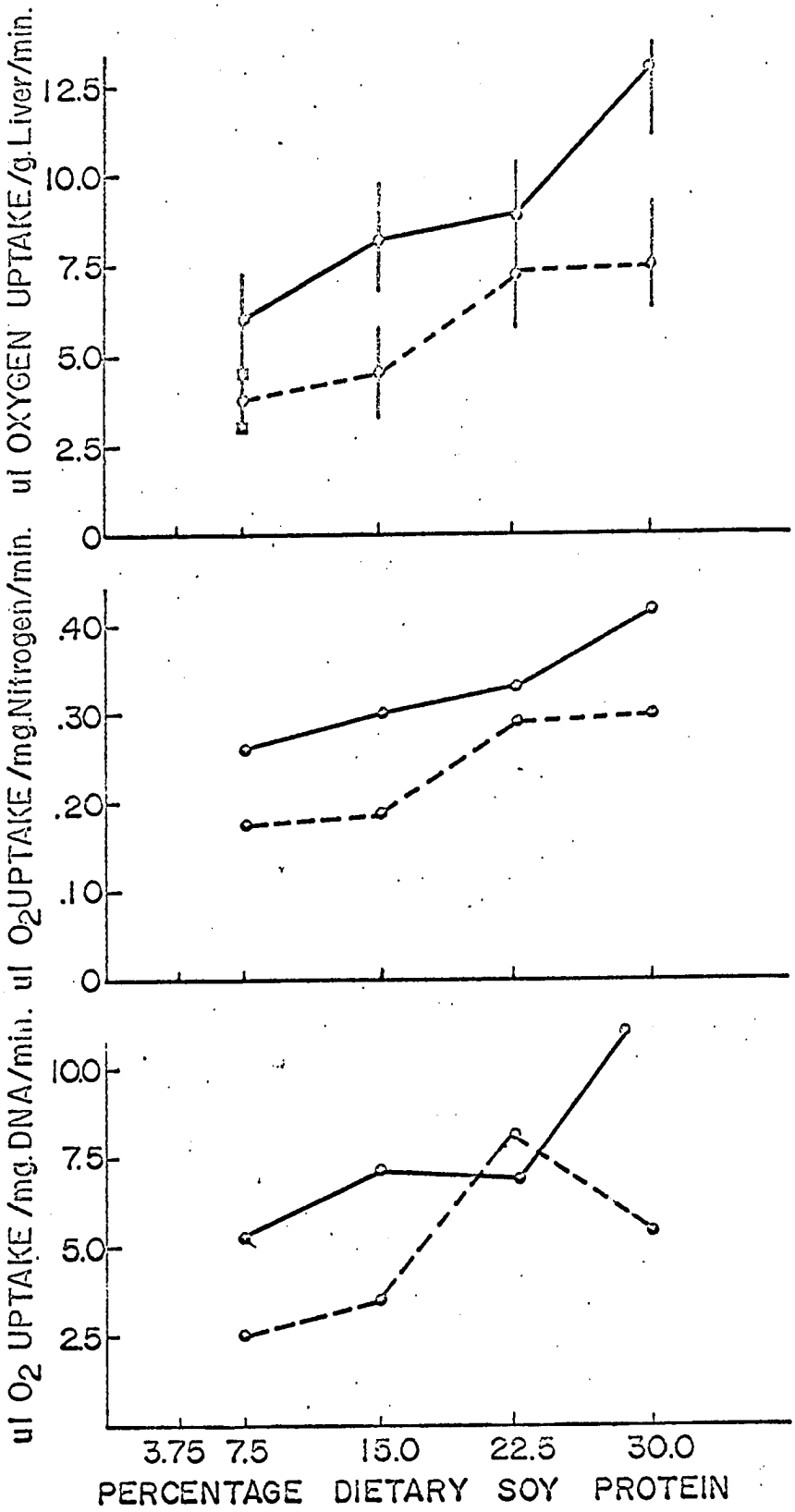


Figure 27. Mean hepatic FAO-16 per mg nitrogen in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiment 3-A.

Figure 28. Mean hepatic FAO-16 per mg DNA in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiment 3-A.


ul OXYGEN UPTAKE/g.Liver/min.
12.5
10.0
7.5
5.0
2.5
0
ul O2UPTAKE/mg.Nitrogen/min.
.40
.30
.20
.10
0
ul O2 UPTAKE/mg.DNA/min.
10.0
7.5
5.0
2.5
3.75
7.5
15.0
22.5
30.0
PERCENTAGE DIETARY SOY PROTEIN

nitrogen, or DNA (Table 45, Figures 26, 27, 28). Mean value of FAO-16, relative to wet weight or DNA, for group SM 30 was significantly larger than those of other groups fed supplemented diets (Table 47). However, mean FAO-16/N ratios associated with methionine supplementation were not different.

Generally, the addition of methionine to protein increased the concentrations of FAO-16 ($P < 0.05$) (Table 48), even though individual comparisons of isonitrogenous diets showed a statistically significant effect of supplementation only at the 30% level of protein intake.

None of the groups fed soy protein had FAO-16 activity values comparable to that found in stock rats (Table 45). The response of Group SM 30 exceeded those of other experimental groups, but was significantly less than that of stock controls. On the other hand, 3.75% control protein diets did not stimulate a response different from that obtained with 7.5% protein diets.

Table 48. Results of Duncan's Test on the effect of methionine on FAO-16 activity[a] - Experiment 3A

% protein	7.5		15.0		22.5		30.0	
Group	S	SM	S	SM	S	SM	S	SM
FAO-16/g	3.7	6.0	4.5	8.3	7.4	8.7	7.5	13.2
P<.01	----------		----------		----------		----------	
P<.05	----------		----------		----------		----------	
FAO-16/N	.18	.26	.19	.30	.29	.33	.30	.42
P<.01	----------		----------		----------		----------	
P<.05	----------		----------		----------		----------	
FAO-16/DNA	2.5	5.2	3.5	7.4	8.2	6.4	5.5	12.6
P<.01	----------		----------		----------		----------	
P<.05	----------		----------		----------		----------	

[a]Any two means not underscored by the same line are significantly different.

DISCUSSION

In order that cellular processes may proceed optimally in mammals, numerous mechanisms exist for maintaining a constant internal environment. Severe dietary deprivations may result in failure of the external environment to provide essential nutrients for replenishment of the internal environment leading to aberrations in cellular functions (Olson, et al., 1958). The extent of changes in nutrient concentrations and of alterations in cellular metabolic processes is influenced in part by duration and degree of nutrient deprivation as well as by growth rate of the animal.

In the present study, evaluations of protein nutriture of young, rapidly growing rats fed varying quantity and quality of soy proteins were made by assessment of the growth rate as well as by determinations of liver nitrogen, lipids, nucleic acids and selected enzyme systems. Alterations in these parameters do not change at the same rate and mechanisms involved are not necessarily interrelated. For these reasons, discussion of the observations made in this study will be divided into several topics: growth, nucleic acids, lipids, and enzyme systems.

Growth

Growth depression of young animals fed low protein diets has been observed by many investigators, too numerous to cite individually. Depending on the nutritive value of the protein under study, dietary concentrations of 15 to 30% protein produced optimum weight gains for many proteins of practical interest. In Experiments 2 and 3 of the present study, rats fed 3.75 and 7.5% unsupplemented soy protein lost weight during the ex-

perimental period. Supplementing the 3.75% protein level with the selected concentration of methionine did not improve the diet sufficiently to evoke a response in growth, but supplementing the 7.5% soy protein diet with methionine did stimulate a moderate growth response.

The growth depression seen with the 3.75% soy protein diet without methionine could probably be ascribed predominately to a lack of total nitrogen available to the animal, and secondly to an unfavorable amino acid pattern. No noticeable changes in body weight were detected when the limiting amino acid of soy protein, methionine, was added to the 3.75% protein diet, nor when the quantity of dietary soy protein was increased to 7.5%. However, a growth response was seen when the 7.5% soy diet was supplemented with methionine indicating that the pattern of amino acids as well as the quantity of nitrogen in diet SM 7.5 was sufficient to induce some weight gain response. This was achieved without an increase in the energy value of the diet since the food intakes of the 2 groups fed 7.5% protein were similar. Consequently, the failure of group S 7.5 to gain weight may be attributed partially to a methionine deficiency which was overcome by adding this amino acid to the diet.

Increasing the amounts of unsupplemented soy protein from 7.5 to 30% by increments of 7.5% was associated with progressive gains in body weights. When the final body weights of animals were plotted against dietary protein a nearly linear relationship was apparent. These results disagree with data reported by Muramatsu and Ashida (1963). These investigators compared the growth rate of young male rats fed 10 to 40% soy protein and found maximum weight gain with 20% soy protein after 15 days. Gains seen with 30 or 40% protein diets did not differ from those with 20% protein

rations. With diets containing 10% protein, the growth response was about 60% of that observed when 20% protein was given. In the present study in Experiment 3, a curvilinear response was apparent only in animals fed soy diets with added methionine, since with 15% supplemented soy protein a plateau in growth rate was reached. However, the growth response obtained with high levels of supplemented soy diets cannot be considered optimum since mean body weights of SM 15, 22.5 and 30 were less than those fed a stock diet.

The plateau in growth rate could be attributed to one or more causative factors. One may be the presence of residual amounts of inhibitor. According to the manufacturer, the soy protein used in the present study had been subjected to heat treatment at 80°C for 30 minutes. The temperature used was 30 degrees below that employed by Parsons (1943) who had obtained optimum protein efficiency when soy flour was autoclaved at 110°C for 30 minutes. The possibility that, with relatively large quantities of insufficiently treated protein in the diet, residual amounts of inhibitor had exerted a growth depressing effect may have to be considered.

Secondly one may question whether the methionine level was adequate. Diets SM 22.5, SM 30 and S 30 were calculated to contain sufficient amounts of the limiting amino acid to exceed the NRC recommendation for the rat (Table 49). Also, though the highest level of protein in Experiment 1 was only 15%, beneficial effects on growth rate could not be attributed to feeding 0.8% over 0.2% methionine. These results confirmed earlier observations in this laboratory which had shown that animals on 15% soy protein diets, with 0.8% and 0.2% methionine supplements (Davidson,

Table 49. Calculated % methionine and cystine of experimental diets - Experiment 3

Group	%
S 3.75	0.08
S 7.5	0.16
S 15.0	0.30
S 22.5	0.45
S 30	0.90
SM 3.75	0.18
SM 7.5	0.36
SM 15.0	0.70
SM 22.5	1.04
SM 30.0	1.40
NRC[a] allowance of % methionine and cystine/100 g diet = 0.6	
α protein[b] contains 1% methionine and 1.1% cystine	

[a]Source: National Academy of Sciences (1962).

[b]Nutritional Biochemical Corporations, Cleveland, Ohio. Analysis of α protein. Private communication. 1965

1963) gained at similar rates.

Finally growth inhibition may be related to the observation that supplementation of an imbalanced protein by the first limiting amino acid may precipitate a deficiency of the second limiting amino acid (Harper, et al., 1953). With high levels of supplemented protein the supply of a second limiting amino acid, either lysine or threonine, may have become insufficient.

Nevertheless, though additional factors seem to play a part, the supplementation of soy protein diets did improve the growth rate of young rats. The addition of cystine to 10% soy protein diets enhanced the growth of weanling rats (Mitchell and Smuts, 1932). Later methionine, rather than cystine, was demonstrated to be the indispensable amino acid (Womack, et al., 1937) of soy protein; in the investigation by Mitchell and Smuts (1932) the soy diet with additional cystine may have been effective in promoting growth because cystine spared methionine.

Comparison of the effects of supplementing 15% soy protein with its limiting amino acid showed that weight gain of animals receiving the amino acid supplement was three times greater than that of animals not fed supplemental methionine (Davidson, 1963). These results were confirmed in the present study where animals on comparable diets also showed a three-fold difference in weight gain.

Dietary protein, hepatic lipids and fatty acid oxidase

Previous investigations in this laboratory had demonstrated the existence of an inverse relationship between the amounts of hepatic lipids and the activity of FAO-8 in weanling rats (Crenshaw, 1962; Davidson, 1963). Lipid accumulation was induced by diets composed of 9% casein or gluten (Crenshaw, 1962) or of 15% protein based on combinations of soy and/or lactalbumin (Davidson, 1963).

In the present study, hepatic lipid infiltration in animals maintained on marginal quantities of soy protein was anticipated. Since estimation of FAO-8 activity associated with the selected levels of dietary soy protein could contribute insight into the mechanism responsible for

hepatic lipid infiltration, FAO activities were determined, using either a short or a long chain fatty acid as the substrate.

Although the long chain fatty acids are found in larger concentrations in mammalian tissue than the short chain fatty acids, investigators have utilized octanoate as a substrate in FAO assays. The relative insolubility of the long chain fatty acids and their inhibitory effect on oxidative processes when the acids are present in excessive concentrations have limited their use for in vitro studies. However, the in vitro oxidation of the two types of fatty acids may follow different pathways since in isolated rat liver mitochondria the end product of oxidation of long chain fatty acids was primarily carbon dioxide, while that for short chain fatty acids was acetoacetate (Kennedy and Lehninger, 1950). Recently, the work of Fritz (1963) demonstrated that the inhibitory effect of the long chain fatty acids could be overcome by attaching the fatty acid to a carrier, such as carnitine. This modification of the FAO-16 assay was carried out on a limited number of rats in Experiment 3-A.

The activity of FAO-16 using palmitylcarnitine as a substrate could not be related to hepatic lipid concentration ($r = -.21$) but was related to protein intake (Table 46). Under conditions of this experiment, the rise in FAO-16 activity and the decline in lipid concentration appear to be independent results of changes in protein intake.

Over the spectrum of protein concentrations tested, hepatic lipids generally declined with increasing unsupplemented protein intakes. Therefore, hepatic lipid infiltration was evident with diets containing low levels of unsupplemented soy protein, but the highest lipid concentrations

were not consistently associated with the lowest protein intakes. Data from Experiment 3 gave a curvilinear response when lipid concentration was plotted against unsupplemented soy protein (Figure 5). Below 15% unsupplemented protein, fatty infiltration was apparent, but not as markedly as with the 15% protein diet. Further addition of protein to 22.5 and 30% caused the lipid value to decline.

Examination of Figures 5, 6 suggested that a peak lipid value could have occurred in both experiments 2 and 3 with a range somewhere between 7.5 and 15% protein. Several indices studied did indicate a transition from an acute to a mild protein deficiency between these two levels. For instance, significant changes were noted in SDH activity as well as in growth rate as the concentrations of unsupplemented protein was raised from 7.5 to 15%. These data suggested that the regulation of hepatic lipids may be dependent on protein concentrations, but that the quantity of protein available for this purpose may be modified by systems of priorities involving demands made on the amino acid pool for growth and enzyme activities.

Hepatic lipid values were low among groups receiving methionine supplemented soy protein over the whole range of protein from 7.5 to 30%. Lipid concentrations associated with diets SM 22.5 and SM 30 as well as with diet S 30 were near stock control values.

The data illustrate two points: 1) the effectiveness of protein as a lipotropic agent was apparent as the soy protein content of the diet was increased and 2) the effectiveness of extra methionine as a lipotropic agentwas evident at most levels of soy protein, but disappeared

when excessive amounts of protein were consumed. Whether the lipotropic effect associated with large amounts of soy protein was due to the relatively high level of methionine supplied as part of the soy protein or to effects unrelated to the methionine content of diets cannot be discerned from the present data.

The reason for hepatic lipid infiltration in young female rats fed marginal levels of soy protein is not known. Soy protein is low in choline and methionine (Olson, et al., 1958). In this study supplements of water-soluble vitamins including choline were given, making the total choline supply from both exogenous and endogenous sources theoretically adequate. Yet on the basis of the synergistic relationship between methionine and choline (duVigneaud, et al., 1939; 1941) a secondary choline deficiency may have existed at low levels of unsupplemented soy protein, caused by limited choline biosynthesis from methionine. The fact that lipid infiltration could be prevented by the inclusion of methionine into diets SM 7.5 and SM 15 lends support to the existence of a conditioned choline deficiency.

Another cause for fatty infiltration may be an imbalance of essential amino acids supplied by soy. Improvement in growth response with the incorporation of methionine at all but the highest level of soy protein intake suggested this possibility. Thus, even though supplemental methionine may have alleviated a marginal choline deficiency, the amino acid was also beneficial for functions related to protein synthesis.

The type of fatty livers induced by choline deficiency seems to differ from that associated with an imbalance of amino acids. Histological studies have revealed the presence of large quantities of lipids with a

centrolobular distribution in choline deficiency compared with a relatively limited degree of peripheral lipid infiltration in amino acid deficiencies (Nino-Herrera, et al. 1954). In the present study, data on lipid distribution in microscopic units are not available. Lipid concentration was not high compared to those encountered in choline deficiency, suggesting that the fatty livers observed were probably not solely due to a secondary shortage of choline.

The mechanisms through which lipotropic factors function need clarification. The possibility of a defect in lipid release processes in choline-deficient fatty livers has been suggested. Olson and associates (1958) reported that for most dietary treatments involving choline deficiencies the marked hypolipemia and excessive hepatic lipid accumulation were prevented by the administration of choline. This suggested that the lesion in choline deficiency was an impairment in release of hepatic lipids resulting in low serum lipoprotein levels and high hepatic lipid concentrations. Failure of lipid release may also occur in methionine deficiency. Relatively low serum cholesterol and phospholipid levels in methionine-deficient female rats with fatty livers led Lyman and associates (1964) to postulate that impaired transport of lipid from liver to the blood contributed to the accumulation of hepatic lipids.

Other mechanisms have also been implicated. Dianzani (1957) observed an accumulation of reduced relative to oxidized pyridine nucleotides in choline-deficient rats. Since reduced pyridine nucleotides are used for synthesis of fatty acids, a condition favorable for synthesizing lipids exists in choline deficiency.

Attempts to locate the metabolic defect in threonine-deficient animals by using ^{14}C-acetate suggested that an increased rate of fatty acid synthesis was the causative factor (Yoshida and Harper, 1960). However, decreased fatty acid oxidase activity and altered concentrations of numerous cofactors of the fatty acid oxidase system led Arata and associates (1964) to hypothesize that a primary defect in the electron transport system led to the observed lesions.

In Experiment 3, FAO-8 activity was inversely related to lipids ($r = -.41$). Although the correlation coefficient was highly significant, the relationship did not hold at the 7.5% protein level (Figures 5,23). At this intake animals failed to grow, showed low levels of FAO-8 activity, and also lower amounts of lipid than group S 15. Beveridge and coworkers (1945) concluded from their data that in protein insufficiency where growth is inhibited some protein may be diverted towards control of lipid deposition. If similar mechanisms were operating in rats fed limited quantities of soy protein, low FAO-8 activity could be associated with hepatic lipid concentrations which are below those predicted from the regression line. The negative relationship between FAO-8 activity and lipid levels was clear cut when the protein intake was between 15 and 30%. Presumably at these concentrations of dietary proteins, sufficient quantities of protein were provided for regulation of hepatic lipid metabolism as well as for growth.

The results of the present study supported the existence of an inverse relationship between FAO-8 and lipid concentration, but the data do not allow deductions as to a causative relationship between the rate of oxidation of the fatty acids and the quantity of lipids accumulated in the liver.

Perhaps concurrent studies on serum lipoprotein levels or rate of hepatic lipid synthesis would cast further light on the regulation of lipid deposition in animals fed soy protein diets.

Ribonucleic acid, deoxyribonucleic acid and ribonuclease

The usefulness of the growth response for the evaluation of protein nutriture is limited by the existence of a plateau in growth rate which may occur at high levels of protein intake, as exemplified in the SM series of the present study. In search for parameters which might detect subtle changes related to dietary alterations, the possibility of modifications of liver RNA by variations in protein intake has been explored. It was assumed that metabolism of this nucleic acid would reflect the extent of protein biosynthesis. Since DNA has been used as an index of the number of cells, the ratio of RNA/DNA might estimate changes in RNA concentration on the cellular level.

Under conditions of this experiment, DNA per g hepatic tissue was not changed by dietary alterations, confirming results that DNA content appeared independent of nitrogen intake (Allison, et al., 1962). Under similar dietary conditions, RNA concentration was not altered significantly. Although nutritional variations did not precipitate differences in RNA relative to DNA based on regression equations, the treatments applied have occasionally produced differences between mean ratios of adjacent groups in a series. These differences may be in part due to the fact that a ratio would magnify variations which are statistically insignificant. For example, the concentration of DNA in group SM 15 appears relatively low, although it does not differ significantly from other values in the

series. RNA values for SM 15 and SM 22.5 were similar but the RNA/DNA ratio of these 2 groups were statistically different. Assuming the differences were not artifacts of computations of ratios, values were similar until the 22.5% protein diet was given. At this point, a significant rise in RNA concentration relative to DNA occurred. Allison and associates (1962) reported progressive enhancement of RNA/DNA as the nitrogen intake was changed over a range from 0 to 25% protein. Relatively larger increases in RNA/DNA ratios were associated with protein sources such as egg or casein as compared to wheat gluten or cottonseed flour, indicating that the degree of change was modified by the quality of the protein.

In the supplemented series of the present study, a nearly significant increase in RNA/DNA ratio was seen when the supplemented soy protein was elevated from 7.5 to 15%, with a significant drop in values with diet SM 22.5. It appears that a peak value for RNA/DNA was reached at a protein concentration which was lower in the supplemented than in the unsupplemented series.

Regression equations relating isonitrogenous diets revealed that additional methionine did not produce changes in the RNA/DNA ratio. Banks (1964) reported that RNA/DNA ratios were not altered by supplementation of wheat gluten with lysine although changes in RNA/DNA ratios were seen when a combination of wheat gluten and casein was used. These observations indicated that changes in the RNA/DNA ratio depended upon the adequacy of the newly created pattern of amino acids. Thus, the possibility exists that the addition of methionine to soy protein may not have influenced protein biosynthesis in the livers because the improved amino acid mixture

was used primarily for growth of tissue other than liver. In the present study, body weights of supplemented groups were greater than those of unsupplemented rats indicating increases in extrahepatic components.

RNA was examined relative to nitrogen because of the suggestion that this ratio may give an index of the rate of protein biosynthesis. In the present study, RNA/N ratios decreased with graded amounts of protein and incorporation of methionine, since under these conditions, the nitrogen content of livers had increased while RNA concentration had not varied. When the concentration of dietary protein was less than 15%, no differences in mean RNA/N ratios were detected in series S or SM.

The quantity of soy protein tested ranged from inadequate to excessive levels. Generally, with liberal protein intakes, there was a trend toward lower RNA/DNA and RNA/N ratios than with moderate levels of protein in the diet. The significance of the relatively low ratios at high protein intakes is not clear.

In the case of the RNA/N ratio, the lowering of the value was due to the increased concentration of nitrogen. If higher nitrogen concentration indicated that more protein was synthesized, one would also expect more RNA. However, determinations of total hepatic nitrogen concentration do not give the information necessary to differentiate between nitrogen fractions essential for optimum function and those expendable, or labile. The labile fraction may be reversibly depleted during conditions of nutritional stress without apparent effect on function (Allison, 1964). For example protein depletion of adult rats produced an 85% decrease in hepatic xanthine oxidase activity without apparent effect on uric acid

metabolism (Bass, et al., 1950). It is possible that the liver of rats on high soy protein diets had accumulated nitrogen which could be readily released without effect on hepatic function.

There are indications that the rate of hepatic nitrogen turnover is high when excessive amounts of dietary protein are consumed. The rise in GPT activity with increases in protein intake found in this study, as well as in that of Schimke (1962) and the concurrent increase in urea cycle enzyme activity reported in the latter, point to the existence of a high rate of nitrogen catabolism without demonstrable malfunction of hepatic processes. Under these conditions RNA would not respond with an increase, while relatively large amounts of nitrogen from the dietary protein would be metabolized in the liver by pathways not involving protein synthesis, adding to the total nitrogen concentration of this organ. The resulting RNA/N ratio would be relatively low, as observed in this study.

Investigations comparing protein free and protein containing diets had demonstrated greater values of RNA in animals fed diets containing nitrogen (Munro and Clark, 1960). Although total RNA had been relatively low in rats fed protein free diets, incorporation studies with ^{32}P suggested that the rate of synthesis of this nucleic acid was actually increased. Furthermore, only a certain fraction of RNA, located in the nucleus, was synthesized more rapidly in protein depletion. Production of this same fraction was suppressed with realimentation (Girija, 1965). Since the rate of nuclear RNA synthesis was actually increased in protein depletion, Munro and Clark (1960) suggested that the depression of RNA

levels may have occurred through breakdown of RNA. Allison and associates (1961) found similarly low levels of RNA in protein deficiency, but extended their studies to include RNAse determinations and reported an inverse relationship between RNA and RNAse. On the basis of these results the activity of RNAse was determined in the present study.

Although the activity of RNAse per g tissue and per mg DNA did not change with dietary variations, the RNAse activity, relative to nitrogen, decreased as the quantity of dietary protein increased. Also, adding methionine reduced the RNAse/N ratio further. In Experiment 1, the control group fed a protein-free ration showed considerably greater RNAse activity per g of liver than groups fed 7.5 and 15% protein. Thus, in the present study, the effect of a frank protein deficiency on RNAse activity was clear, but the influence of graded amounts of soy protein on RNAse was evident only when activity was expressed on the basis of nitrogen. However, since the RNA/g liver did not change, one would not expect changes in RNAse activity if the theory of an inverse relationship between RNA and RNAse holds true.

The response of RNAse to alteration in protein intake has been investigated by several laboratories, but the results are conflicting (Table 50). Experiments of Allison and co-workers (1961, 1962, 1964) demonstrated a distinctly negative relationship between RNAse activity and RNA per g liver. As the protein intake was increased, RNA concentration increased, and RNAse activity decreased. Furthermore, these authors were able to show that the quantity and quality of protein definitely played an important role in determining the RNAse activity as well as concentration of RNA. That

protein-depletion caused an increase in RNAse activity was also observed by Girija and co-workers (1965). In addition, these investigators failed to obtain appreciable differences in susceptibility of fractionated RNA toward RNAse activity *in vitro*. Since there was a concomitant decrease in RNAse inhibitor in protein depleted rats, the increased RNAse activity was attributed to the instability of the inhibitor.

Results from other laboratories have failed to confirm an inverse relationship between dietary protein and RNAse activity. No differences in RNAse per mg nitrogen and an increase in RNAse per g liver were observed in young animals fed graded quantities of casein (Matsuo, *et al.*, 1966). Analyses of the supernatant, microsomal and mitochondrial fractions of livers of protein-depleted, pair-fed, or control weanling rats did not reveal any differences in acid or alkaline RNAse activity (Corcos Benedetti, *et al.*, 1966).

There are several factors which may contribute to discrepancies observed in the response of hepatic RNAse activity to alterations in dietary proteins. One may relate to the existence of more than one species of RNAse. At least two pH values are known at which optimum activity of RNAse has been observed, thus giving rise to the pH 5.8 (acid) and the pH 7.8 (alkaline) RNAses. Allison (1964) stated that the acid RNAse reflected alterations in dietary protein but the alkaline RNAse was stable under these conditions. In most experiments cited, the pH of the reaction

media lay between 7.4 and 7.8, which would indicated that investigators did not attempt to differentiate between the two species. The second factor may be related to the purity of the substrate used in the enzyme assay. Matsuo and coworkers (1966) suggested that the response obtained in their experiment could have been influenced by the use of a substrate which was not highly purified. Brody (1957) proposed that early experiments did not show alterations in RNAse activity because the substrate had not been purified sufficiently to remove the RNAse inhibitor. If lack of purity of the substrate was the primary reason for the results obtained by Matsuo and coworkers (1966), it would probably be for reasons other than the presence of inhibitors since they reported an increase in concentration of RNAse with increases in dietary protein. The third factor responsible for discrepancies in results between laboratories may be differences in methodology. Both methods cited (Table 50) involve the release of acid soluble nucleotides upon incubation. The reaction is stopped by trichloracetic acid (Brody, 1957) and by acid-alcohol (Roth, 1959). Brody suggested the use of lanthanum acetate which should facilitate the precipitation of the excessive RNA. However, in some of the experiments which were cited in this discussion the investigators modified the method of Roth by inclusion of heavy metals such as lanthanum or uranyl acetate.

Corcos Benedetti and coworkers (1965) added a surface active agent to the mitochondrial and microsomal fractions. The function of the detergent was to increase the permeability of membranes. Since no differences in the three fractions, mitochondrial, microsomal, and supernatant, could be demonstrated, the authors suggested that protein deficiency affected an increase in membrane permeability similar to that seen with detergent.

Table 50. Effects of increasing amounts dietary protein on concentrations of hepatic ribonuclease in young rats

Reference	Days exp. period	Year	Strain	Av. initial wt. of rat	Sex	Diet	Method of analysis	pH of reaction media	Effect	Remarks
Zigman and Allison	28	1959	Wistar	Weanling	Male		Brody	7.4	D[a]	
Allison, Wannemacher, Parmer and Gomez	28	1961	Wistar	100-150	Male		Brody	7.4	D	
Allison, Wannemacher, Banks, Wunner, Gomez-Brenes	28	1962	Wistar	Weanling	Male	9,12,18,25% egg albumin, casein, cotton-seed flour or wheat gluten	Brody	7.4	D	
Girija	21	1965	Wistar	125-150	Male	0,18% casein	Brody	7.8	D	
Matsuo	15	1966	Donrya	79	Male	0,5,10, 20,30% casein	Roth	7.7	I[b](per g tissue) NC[c](per mg N)	Used impure substrate

[a] D decrease.

[b] I increase.

[c] NC = no change.

Table 50. (Continued)

Reference	Days exp. period	Year	Strain	Av. initial wt. of rat	Sex	Diet	Method of analysis	pH of reaction media	Effect	Remarks
Corcos Benedetti	30	1966				0,20% casein, 20% pair fed	Roth	7.8 and 5.6	NC	Added surface active agent
Present study	28	1966	Wistar	45-60	Female	7.5,15, 22.5, 30%	Roth	7.4	D(per mg N) NC(per g liver)	
		1963	Wistar	50	Female	0,75,15% Soy or lactalbumin	Roth	7.4	D 0-7.5% NC 7.5 to 15%	

Unfortunately, the study did not include measurements of activity without added detergent.

In the present study, results from Experiment 1 confirm the effect of protein deficiency with respect to RNAse as seen by Allison (1962). With inclusion of protein in the diet, no differences in RNAse values were detected in response to the quantity or quality of protein. This finding is contrary to Allison's data and more in agreement with reports by Corcos Benedetti and associates (1966) and Matsuo and coworkers (1966). Both groups had used casein containing diets. It should be kept in mind, however, that the protein used for this study, soy protein, was not of high biological value, and that the animal's adjustment to such a protein may differ from adaptive responses made to a nutritionally high quality protein.

Selected hepatic enzymes and dietary proteins

The activities of the enzyme systems selected for the present study are expressed on the basis of liver weight, DNA content, or nitrogen concentration because no single method is available which adequately describes the interrelationship of liver components after dietary treatment. By expressing activity on the basis of wet weight alone the degree of dilution of a constituent by other components, especially by lipid and water is not indicated. Activity on the basis of DNA would relate activity to the number of cells, since cellular content of DNA does not appear to be significantly altered by dietary deprivation in adult rats (Campbell and Kosterlitz, 1948). However, the age of the experimental animal may be important as indicated by investigations with immature rats. Nuclei from livers of growing male rats fed 12% protein diets contained

almost 20% more DNA per nucleus than those from control animals maintained on 26% protein diets (Ely and Ross, 1951). This observation was not confirmed by Allison and coworkers (1962) who reported that weanling male rats with nitrogen intakes of 2 to 8 g for 28 days did not show changes in mg DNA per g liver. In the present study, the concentration of DNA was also similar regardless of the dietary treatment; the number of nuclei per unit was not determined and was assumed to be unaffected by diet.

Liver nitrogen has also been used as a basis of comparison of other liver components since it may serve as an index of functional tissue. However, the classical studies of Addis and associates (1936) which illustrated that quantity of hepatic nitrogen was modified by protein intake, suggested that a certain fraction of liver nitrogen was labile.

Analyses of enzyme systems selected for the present study demonstrated that the activities of SDH, FAO-8, FAO-16 and GPT per g liver increased as the protein in the diet was elevated. Furthermore, all these enzymes, with the exception of GPT, showed relatively greater activity when methionine was incorporated into the diets. These alterations in enzyme activity may reflect adaptations by the animals to nutritional variations. The regulation may involve hormonal factors (Tepperman and Tepperman, 1960), but the details of the mechanism by which alterations in dietary constituents exert control over hepatic enzyme activity are essentially unknown. However, studies in bacterial systems indicate that the rate of synthesis and activation of preformed enzymes may play a role in the control of enzyme capacity.

That synthesis of enzymes may be subject to double genetic control was suggested by Jacob and Monod (1961). They postulated that one gene

determines the structural organization of the enzymic protein and the second gene controls the rate of synthesis through cytoplasmic components. Accordingly, enzyme formation depends on a supply of amino acids which will be organized according to determinants of the first gene and on a supply of compounds which induce or repress synthesis under control of the second gene.

Data on body weight changes have indicated that the nutritive value of diets containing 7.5% soy protein could be improved by either changing the amino acid pattern with additional methionine or by increasing the total amino acid supply with additional soy protein. The activities of several hepatic enzyme systems in group S 7.5 was greatly reduced in comparison to those of groups receiving more protein or additional methionine. This trend was apparent with respect to the activities of the SDH and FAO-8 systems as well as to concentrations of hepatic nitrogen. The data could be interpreted to mean that the improvement in the quantity or balance of the amino acids permitted the structural gene to be expressed to a fuller potential.

Control of enzyme synthesis is also suggested to occur at the post gene level. For instance, the rate of conversion of an apoenzyme of tryptophan pyrrolase to an active holoenzyme was determined by the quantity of tryptophan. The subsequent accelerated protein synthesis increased the total enzyme protein (Greengard and Feigelson, 1961).

In the present study, greater activity of FAO-8, FAO-16, SDH and GPT were associated with higher levels of dietary protein, suggesting that animals may have adjusted to a range of protein intakes by adapting the

level of enzyme activity. The most marked differences in activity were observed when groups S 7.5 and S 30 were compared. These results obtained with soy protein parallel those of Muramatsu and Ashida (1962) who used casein as a protein source and found that graded quantities of protein stimulated increases in hepatic SDH and GPT activities.

Since the SDH system is involved in energy production while the GPT system is required for amino acid metabolism, the responses of these 2 enzymes to gradations in protein intake were of particular interest. SDH value of group S 22.5 was less than that of group S 30, but mean GPT concentrations differed only when groups S 7.5 and S 30 were compared. Thus, GPT per g tissue appears to be stabilized over a wider range of protein intake than SDH.

The growth rates of rats fed unsupplemented soy diets had been linear over the range of protein intakes from 7.5 to 30%. The pattern of response of hepatic SDH and GPT systems nearly paralleled the growth curve (Figure 29). All three parameters showed relatively similar permutations with stepwise increments of unsupplemented protein in the diet. Growth data illustrated that the 7.5% unsupplemented soy protein diet was inadequate for weight maintenance. Since anabolic and catabolic processes at this level of protein intake were probably at a minimum, low levels of enzyme activity might be expected.

With each increment of soy protein from 7.5 to 30%, progressively more of the amino acid and energy requirements of the animals were satisfied. This was mirrored by increases in hepatic SDH and GPT activity as well as rate of growth. Under conditions of this experiment, maximum

Figure 29. Mean body weights, mean unit GPT/g liver, and mean mg formazan/g liver of rats fed varying quantities of soy protein without methionine (diet S). Experiment 3.

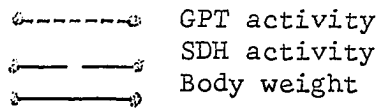
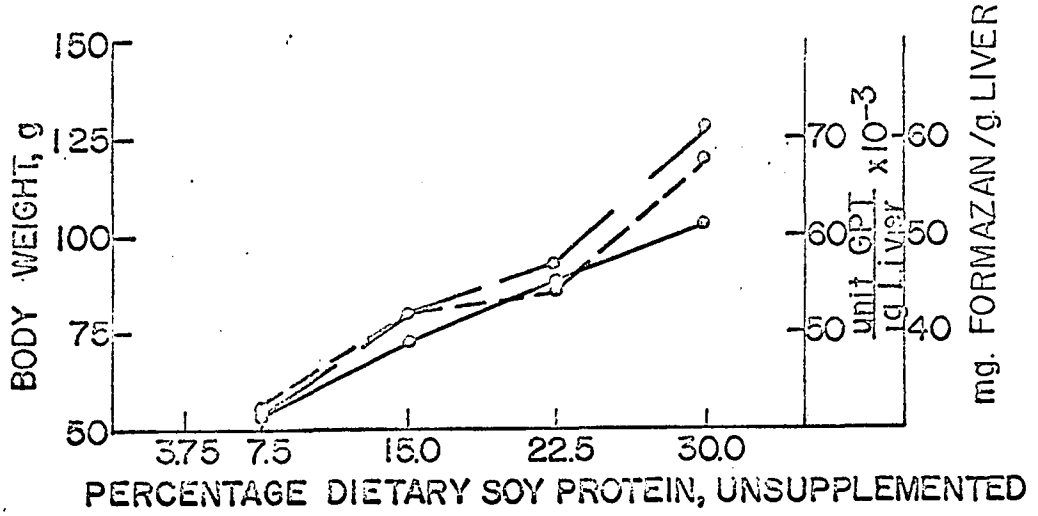
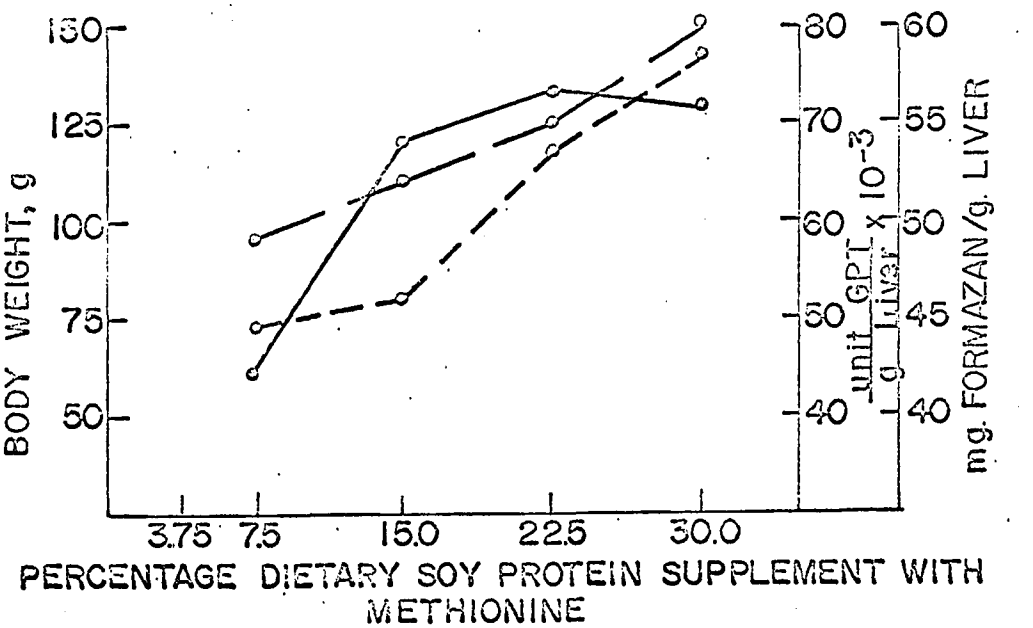


Figure 30. Mean body weights, mean unit GPT/g liver, and mean mg formazan/g liver of rats fed varying quantities of soy protein with methionine (diets SM). Experiment 3.


BODY WEIGHT, g
150
125
100
75
50
3.75
7.5
15.0
22.5
30.0
unit GPT x 10⁻³ / g Liver
70
60
50
60
50
40
mg. FORMAZAN/g. LIVER
PERCENTAGE DIETARY SOY PROTEIN, UNSUPPLEMENTED


BODY WEIGHT, g
150
125
100
75
50
3.75
7.5
15.0
22.5
30.0
unit GPT x 10⁻³ / g Liver
80
70
60
50
40
60
55
50
45
40
mg. FORMAZAN/g. LIVER
PERCENTAGE DIETARY SOY PROTEIN SUPPLEMENT WITH METHIONINE

activity of these 2 enzymes and greatest weight gains were associated with 30% protein in the unsupplemented series. However, the SDH and GPT activity of groups S 22.5 and S 30 as well as the GPT concentration of group S 15 were comparable to those of stock controls, indicating that unsupplemented soy protein in sufficient quantities will supply amino acids necessary for maintenance of these enzyme systems at near optimum levels. In contrast, the unsupplemented protein diets were inadequate for optimum growth since body weights of stock animals were considerably higher than those of the unsupplemented groups.

The results for GPT activity in the present study agree with those reported by Muramatsu and Ashida (1962) and Schimke (1962). These investigators reported progressive increases in GPT activity with elevation of dietary casein intakes. On the other hand, Muramatsu and Ashida studied hepatic SDH activity as a function of graded levels of dietary protein from casein (1962), egg albumen, fish meal, soybean protein, and wheat gluten (1963). With the exception of soy protein, the various dietary treatments stimulated responses in hepatic SDH which paralleled changes in growth curves. Soybean protein diets in excess of 15% protein produced a plateau in growth rates while under these conditions SDH activity continued to increase in a linear fashion. With casein, fish meal or egg albumen diets, both growth and SDH activity showed a plateau with high levels of protein, but the points of inflection of the growth and enzyme response curves did not coincide for a given dietary treatment. When graded quantities of wheat gluten were used as the source of dietary protein, both growth rate and enzyme activity continued to increase linearly. These observations suggested that SDH activity was influenced by both

quantity and quality of dietary protein.

As was pointed out previously, progressive increments of unsupplemented dietary soy proteins produced nearly linear increases in the concentration of GPT and SDH. A similar trend was seen in the response of these 2 enzymes when activity was expressed on the basis of DNA, suggesting that the activity of SDH and GPT were increased more rapidly than the number of cells. On the other hand, when these enzymes were related to nitrogen, only SDH showed an increase with elevation of protein intakes. GPT activity per mg nitrogen appeared to be unaltered. Apparently variations in nitrogen intakes induced similar quantitative changes in hepatic protein and GPT. This observation implies that the induction of GPT may be dependent on the amount of hepatic nitrogen.

A plateau in the growth curve was evident when high levels of methionine-supplemented soy protein were fed, but SDH and GPT activities continued to increase under these conditions (Figure 30). GPT activity was not substantially increased until the diet contained 22.5% supplemented protein. Actually improvement of the amino acid pattern of soy by adding methionine had no further effect on GPT activity since activities of comparable groups of the S and SM series were similar. As was indicated previously, variations in the quantity of protein had some influence on the GPT activity, but changes in quality of protein had little effect. Thus, the activity of GPT appears to be related to the total amino acids available and refractory to alterations of the amino acid pattern.

Based on regression equations, SDH activity appeared to be influenced by changes in quantity and quality of proteins. Comparable groups on isonitrogenous diets showed differences in activity though these could

not be verified statistically. However, a marked difference was seen in values for groups S 7.5 and SM 7.5. Thus, the inclusion of additional methionine into soy protein diets was especially effective in raising the SDH concentration at low protein intakes and had relatively little influence on SDH activity in the presence of ample amounts of dietary proteins.

CONCLUSIONS

Assessments of protein nutriture have been described in terms of changes in body and liver weights. Attempts to evaluate more subtle changes led to measurements of hepatic lipid and enzymes, as well as nitrogen and nucleic acids. In order to establish trends induced by changes in quantity and nutritive quality of soy protein diets, variations in these indices are classified into 3 categories; those which 1) increased, 2) were unaffected, and 3) decreased with increasing quantities of soy protein, with or without methionine.

On the basis of regression calculations, most of the indices selected tended to increase with elevation of dietary protein. At intakes of less than 15% unsupplemented soy protein, rats failed to thrive indicating protein insufficiency. Several other indices--activities of FAO-8 or SDH and concentration of nitrogen--showed significantly low values under the same dietary conditions. Increments of dietary soy protein without methionine above 15% produced weight gains, accumulation of nitrogen and trends towards increasing activities of the selected enzymes.

The incorporation of methionine into soy protein diets created a new amino acid pattern which was more suitable for metabolic functions than the unsupplemented soy protein except at the lowest protein intake of 3.75%.

Although adding methionine to 7.5% protein did not affect GPT activity, the addition of the amino acid at this protein level stimulated SDH and FAO-8 activities and allowed for an increase in hepatic nitrogen. Thus, the effect of protein insufficiency could apparently be overcome by elevating the soy protein content or adding methionine to the diet.

The activities of several enzymes were similar to stock control values when the unsupplemented soy protein intake was 30%, but control levels were attained with a lower protein intake of supplemented soy protein. The nutritive value of the soy diet with added methionine was not comparable to stock rations since the growth curve of this series reached a plateau at a weight level below the maximum attainable under conditions of this experiment. The quantity of the amino acid selected for supplementation presumably was neither limiting nor toxic. However, the possibility that the second limiting amino acid may have become relatively deficient for effective utilization of the high concentration of soy protein exist. With intermediate and high levels of supplemented soy diets, overall growth rate was depressed, while the liver continued to respond to increments of soy and methionine. Activity of the three enzymes under discussion as well as hepatic nitrogen concentration continued to increase.

Indices which appeared to be independent of nitrogen intake were concentration of RNA and DNA. The results of DNA analyses confirmed reports that DNA concentration does not reflect changes in protein nutriture. RNA values associated with protein containing diets have been reported to be higher than those seen with protein deficient diets. In the present study, hepatic RNA differences with treatments could not be detected in rats on soy protein containing diets. Indications of greater RNA values with proteins of high biological value have been suggested, but since the biological value of soy protein is not as high as some proteins used by other investigators, it seems that changes in RNA were too

subtle to be detected. Perhaps fractionation of RNA would reveal the effect of soy protein diets on the rate of synthesis of the specific RNA component which is supposed to vary during protein depletion and repletion.

The parameter which generally decreased with additional soy protein was lipid. This observation confirmed reports that proteins exert a lipotropic effect. In addition, the lipotropic effect of methionine was especially prominent when the protein content of the diets was low. Although an inverse relationship between FAO-8 activity and hepatic lipid accumulation was evident when the diet contained more than 15% unsupplemented soy protein a causative relationship between activity of FAO-8 and hepatic lipid concentration could not be deduced from the available data.

Changes in RNAse activity fell into two categories. In the preliminary experiment, the activity associated with protein-deficient diets was definitely greater than that observed with protein-containing diets. In subsequent experiments when the protein-free diet was omitted, differences between RNAse activity were not apparent, except when activity was expressed on the basis of nitrogen in Experiment 3.

Thus, a positive relationship between RNA values and protein intake was not established for soy. Although FAO-8 alterations paralleled variations in the soy protein intake, whether the increase was due to protein or incidental to lipid cannot be discerned from the present data. SDH, FAO-8, and GPT activity responded to elevation in soy protein content. In addition, SDH was also a sensitive index of improvement of the amino acid pattern of soy protein.

SUMMARY

Groups of 9 female weanling rats were maintained on diets of purified soy protein containing 7.5, 15, 22.5, and 30% protein. Similar groups were fed isonitrogenous diets of soy protein supplemented with its limiting amino acid, methionine. Three additional diets, laboratory stock ration, 3.75% soy protein with methionine, and 3.75% soy protein without methionine, served as controls. The concentrations of protein selected were based on observations in preliminary studies which tested 2 levels of protein from lactalbumin or soy protein and varying degree of supplementation with methionine.

At the end of a 28 day experimental period, the animals were sacrificed. Livers were assayed for lipids, nitrogen, RNA and DNA. Further analyses of a series of hepatic enzyme system - SDH, FAO-8, FAO-16, GPT, and RNAse - were made. The activity of enzymes were expressed on the basis of hepatic tissue weight, DNA, or nitrogen.

The trend of response was determined by linear regression and the slopes of the lines were compared by the F test. Duncan's test was used to test differences between group means. Correlation coefficients were used to relate the response of any two parameters to dietary variations.

With diet S 7.5 the animals failed to grow, but with successive increments of dietary soy protein, increases in body weight and hepatic nitrogen concentrations were observed. Under similar dietary conditions, RNA and DNA concentrations did not deviate significantly. Hepatic lipid concentration was high when less than 15% protein diets were fed, and gradually declined as the diets contained progressively larger quantities

of protein.

After the addition of methionine to soy protein, the concentrations of hepatic lipids, RNA, and DNA were not different over the range of protein levels tested, although the nitrogen content increased significantly. As stepwise increments of supplemented proteins were included in the diets, a plateau in growth rate occurred before the weights of the experimental animals had reached those of stock controls.

Comparison of isonitrogenous diets revealed significant increases in body weight and nitrogen concentration as well as a reduction in liver lipid concentration due to the addition of methionine. Under similar dietary conditions, values for RNAse, RNA and DNA did not change.

Changes in activities of selected enzymes - FAO-8, FAO-16, SDH, and GPT - paralleled increasing levels of protein in the diet when regression equations were computed, although mean differences between adjacent groups were usually not detectable. In the unsupplemented series, the increase of protein intake from 7.5 to 15% had a more marked effect on enzymic activities than subsequent additions of dietary protein. This alteration in activities occurred concurrently with resumption of growth indicating a shift from predominantly catabolic to anabolic processes. The mean activities for several enzymes were often not statistically different when protein intake was increased from 15 to 22.5 to 30%, indicating that adaptation may have occurred.

Enzyme activities associated with the inclusion of methionine into isonitrogenous diets suggested that methionine was especially effective in altering responses of animals when the dietary soy protein intake was marginal. In several enzyme systems studied, the feeding of 30% un-

supplemented soy protein diet was sufficient to bring the level of activity to that of controls, while the same level was achieved by feeding 22.5% supplemented soy protein diet.

FAO-8 was inversely related to lipid in the series where fatty infiltration had occurred. However, since changes of other enzyme systems paralleled protein variations, the inverse relationship of FAO-8 activity and lipid concentration may be secondary to primary changes in these two parameters to alterations in protein intake.

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APPENDIX

Table 51. Hepatic enzymes and nucleic acids in young female rats fed varying quantities of soy protein, with and without methionine, for 28 days; values expressed per g liver - Experiment 3

Group	Rat no.	FAO-8 u l	SDH mg	RNAse mg	GPT units	DNA mg	RNA mg
S 7.5	421	25.4	32.3	0.967	51.7	0.97	14.84
	437	27.0	38.4	1.230	39.9	1.06	12.84
	454	21.6	19.0	1.332	29.2	1.32	12.62
	460	8.0	12.7	0.998	39.5	1.16	9.31
	461	23.2	18.7	0.488	41.0	1.13	12.94
	471	8.2	0.6	1.354	34.7	1.44	11.86
	535	39.2	8.3	1.060	24.9	1.70	9.76
	544	24.2	7.6	1.385	38.8	1.12	13.44
	591	6.2	40.2	0.494	76.0	1.34	13.01
Mean		20.3	19.8	1.034	41.7	1.25	12.29
S 15.0	422	33.4	52.3	1.184	41.4	1.03	15.83
	426	34.0	68.7	1.115	54.4	1.69	11.42
	468	45.8	40.5	1.602	48.4	1.47	12.76
	470	22.8	6.9	0.766	57.4	2.24	10.84
	482	47.2	30.0	0.377	52.5	1.34	12.62
	530	32.6	22.4	0.117	45.2	1.23	9.80
	542	53.4	45.9	0.881	44.8	1.00	14.69
	569	33.6	49.4	0.801	74.0	1.34	8.92
	582	27.6	63.6	0.424	55.6	1.38	11.60
Mean		36.7	42.2	0.807	52.6	1.41	12.05
S 22.5	427	35.0	66.1	1.118	61.2	1.23	10.71
	439	49.6	50.8	0.558	27.3	1.05	7.22
	443	38.4	39.8	0.495	44.8	1.03	14.28
	457	64.4	49.2	0.848	45.1	2.35	13.10
	475	36.0	31.2	1.030	30.7	1.66	17.67
	481	51.6	47.2	0.370	77.5	1.06	15.27
	533	44.6	46.3	0.769	45.3	1.62	11.77
	561	32.2	35.4	0.611	43.7	1.31	15.58
	570	32.8	50.6	1.194	107.5	1.37	10.89
Mean		42.7	46.3	0.777	53.7	1.26	12.94
S 30.0	444	66.6	59.0	0.661	58.7	1.33	14.43
	455	72.4	52.5	0.925	45.0	1.44	12.97
	463	48.0	48.7	0.527	57.4	1.64	11.79
	467	40.0	65.2	0.668	39.2	1.64	11.79
	473	64.6	53.8	0.314	37.6	1.28	12.20

Table 51. (Continued)

Group	Rat no.	FAO-S u l	SDH mg	RNAse mg	GPT units	DNA mg	RNA mg
S 30.0 (Continued)							
	532	68.2	46.6	0.900	95.0	2.08	12.81
	563	32.0	73.9	0.993	68.0	0.72	13.98
	584	31.2	85.4	1.506	80.7	1.42	13.34
	589	44.4	68.1	0.904	123.8	1.28	14.67
Mean		51.9	61.5	0.822	67.3	1.41	12.99
SM 7.5	425	33.6	76.1	1.173	26.5	1.24	12.40
	441	46.0	32.2	0.392	54.2	1.72	18.25
	466	88.8	46.5	1.606	47.3	1.45	12.12
	474	40.4	27.0	1.000	46.7	1.40	13.34
	484	11.6	42.8	0.290	48.2	1.63	14.26
	531	43.2	44.9	0.246	45.2	1.91	11.35
	564	33.2	48.5	0.401	71.8	1.34	12.27
	571	46.8	45.0	1.014	51.1	1.08	11.25
	583	3.8	83.2	0.242	52.0	1.53	12.30
Mean		38.6	49.6	0.767	49.2	1.43	13.06
SM 15.0	438	45.6	48.8	0.775	42.5	0.94	8.37
	442	50.4	49.5	0.499	40.3	1.00	18.22
	456	26.2	31.7	0.544	27.7	0.98	10.37
	459	60.4	46.3	0.563	49.3	1.03	13.27
	464	40.4	47.2	0.906	79.5	1.40	12.67
	476	46.6	36.6	1.319	35.4	1.14	13.56
	534	41.6	77.4	0.810	60.0	1.59	11.89
	562	28.8	86.8	0.620	88.6	0.94	13.31
	592	17.4	47.7	0.699	48.2	1.30	15.68
Mean		39.7	52.4	0.748	52.4	1.56	13.04
SM 22.0	423	61.0	60.6	0.744	42.7	1.23	14.86
	453	55.6	48.2	0.544	47.0	1.58	11.80
	462	39.8	52.8	0.882	56.1	2.10	12.80
	465	69.2	51.5	0.810	74.5	1.79	10.71
	472	86.6	28.9	0.929	63.0	1.93	8.12
	529	62.6	62.8	0.405	76.7	1.62	12.14
	543	82.8	56.2	0.618	49.5	1.59	16.54
	581	23.6	80.9	0.760	109.5	2.37	14.67
	590	17.0	56.2	0.754	85.8	1.65	16.16
Mean		55.4	55.3	0.716	67.2	1.76	13.09
SM 30.0	424	45.0	69.2	0.862	78.1	2.06	22.53
	428	58.0	67.5	1.000	107.8	1.54	12.41
	440	53.8	57.2	0.998	58.6	1.05	7.34
	458	56.4	63.7	0.755	95.0	2.46	13.25
	469	62.0	59.8	0.964	20.8	1.14	8.82
	483	63.8	49.2	0.540	68.8	1.20	14.47
	536	60.6	66.2	0.466	105.0	1.78	10.63
	541	56.4	50.5	0.421	79.5	1.16	13.60
	572	53.8	64.1	0.749	80.7	1.15	11.14
Mean		56.6	60.8	0.751	77.1	1.50	12.69

Table 52. Percent hepatic nitrogen and lipids in young female rats fed varying quantities of soy protein, with or without methionine, for 28 days - Experiments 1, 2, 3

Experiment	Diet	Rat no.	Hepatic nitrogen %	Hepatic lipid %
1	PF	10	2.39	10.6±1.2[a]
	L 7.5	10	2.90	11.8±1.5
	L 15	10	3.15	10.3±1.2
	LM 7.5	10	2.53	6.8±.4
	LM 15	10	2.79	6.0±.3
	SM 7.5	10	2.66	10.5±1.3
	SM 15	10	3.19	7.4±0.9
	S4M 7.5	10	2.54	8.1±0.9
	S4M 15	10	3.01	5.5±0.5
	Stock	10	3.17	4.7±0.3
2	S 3.75	10	2.55	14.2±1.5
	S 7.5	10	2.79	17.0±0.7
	S 15	10	2.94	13.3±1.4
	SM 3.75	10	2.81	12.0±1.8
	SM 7.5	10	3.01	9.7±1.2
	SM 15	10	3.26	7.8±0.8
	Stock	10	3.25	4.6±
3	S 3.75	6	2.29	10.7±1.1
	S 7.5	9	2.14	10.9±1.4
	S 15	9	2.30	13.8±1.3
	S 22.5	9	2.60	9.3±1.2
	S 30	9	2.74	6.4±.6
	SM 3.75	6	2.64	5.8±1.4
	SM 7.5	9	2.61	7.4±1.1
	SM 15	9	2.49	7.4±0.7
	SM 22.5	9	3.06	5.3±0.5
	SM 30	9	3.48	5.1±0.4
	Stock	12	3.21	4.2±0.9

[a]Standard error of the mean.

Table 53. Body and liver weights, food intake, hepatic nitrogen, and lipid concentration in young female rats fed varying quantities of soy protein, with and without methionine, for 28 days - Experiment 3

Group	Rat no.	Body wt. g	Liver wt. g	Food intake g	Nitrogen %	Lipid %
S 7.5	421	48	2.45	70	2.20	13.56
	437	44	1.70	131	2.32	5.93
	454	50	2.17	152	2.34	11.83
	460	47	2.10	123	1.98	15.91
	461	44	1.94	177	2.22	6.87
	471	48	2.43	118	1.88	16.31
	535	47	1.77	156	2.17	6.87
	544	49	2.06	145	1.48	13.51
	591	51	2.42	100	2.67	7.03
Mean		48	2.12	140	2.14	10.87
S 15.0	422	60	2.76	132	2.45	11.59
	426	67	3.41	168	2.12	19.03
	468	73	3.08	208	2.30	8.32
	470	68	2.86	142	2.04	16.51
	482	75	3.50	184	2.24	19.70
	530	77	3.23	162	2.20	14.22
	542	60	2.42	114	2.96	10.15
	569	78	3.22	202	2.27	10.61
	582	83	3.37	153	2.14	14.52
Mean		71	3.09	163	2.30	13.85
S 22.5	427	90	4.58	208	2.16	11.35
	439	86	3.14	203	2.77	7.34
	443	92	3.57	212	2.97	8.17
	457	82	2.86	228	2.88	7.89
	475	83	3.71	194	2.46	13.91
	481	97	3.73	242	2.87	6.18
	533	94	3.78	213	2.06	15.97
	561	77	3.36	234	2.44	5.24
	570	88	3.34	163	2.80	7.28
Mean		88	3.56	211	2.60	9.26
S 30.0	444	100	3.93	199	2.71	5.42
	455	109	4.41	250	3.01	4.71
	463	100	4.82	297	2.87	4.67
	467	87	3.31	199	2.74	6.72
	473	115	4.33	254	2.88	10.04

Table 53. (Continued)

Group	Rat no.	Body wt. g	Liver wt. g	Food intake g	Nitrogen %	Lipid %
S 30.0	532	102	3.58	177	2.88	5.80
	563	88	3.50	219	2.22	6.63
	584	91	3.28	220	3.03	4.79
	589	115	5.46	232	2.34	9.13
Mean		101	4.07	227	2.74	6.43
SM 7.5	425	62	2.35	192	2.78	5.19
	441	71	2.83	161	3.14	8.39
	466	70	2.45	168	2.58	9.35
	474	55	2.15	139	2.74	6.15
	484	71	3.40	153	2.18	5.94
	531	64	2.65	156	2.77	10.14
	564	70	3.47	175	2.43	7.06
	571	65	2.32	212	2.40	5.13
	583	61	3.13	175	2.48	9.14
Mean		65	2.75	170	2.61	7.39
SM 15.0	438	108	3.72	210	2.70	8.16
	442	142	4.85	241	3.04	8.14
	456	128	5.81	262	2.02	5.20
	459	116	4.26	243	1.22	4.53
	464	120	4.19	283	2.60	9.57
	476	118	4.27	248	2.58	7.16
	534	131	4.84	246	2.68	7.73
	562	117	4.23	322	3.10	5.12
	592	120	5.63	229	2.44	10.55
Mean		122	4.64	254	2.49	7.35
SM 22.0	423	140	5.55	297	3.14	4.70
	453	124	4.51	276	3.42	4.18
	462	139	4.90	257	3.12	3.72
	465	116	4.07	213	3.10	5.37
	472	123	4.14	221	3.13	4.79
	529	144	5.16	209	2.72	4.03
	543	118	4.13	184	2.56	5.84
	581	105	3.59	260	3.49	6.73
	590	154	5.99	248	2.88	8.46
Mean		129	4.67	241	3.06	5.31
SM 30.0	424	91	3.35	196	4.96	7.45
	428	149	5.39	235	3.60	4.55
	440	127	4.99	306	4.08	4.83
	458	128	5.01	201	3.18	3.97
	469	123	3.65	211	3.22	5.82
	483	126	4.85	238	2.87	4.20
	536	153	6.37	228	2.84	4.97
	541	124	4.61	246	3.20	4.47
	572	145	5.74	246	3.35	5.61
Mean		130	5.00	234	3.48	5.10

Table 54. Body and liver weights, FAO_{16}, hepatic nitrogen, and lipid concentration in young female rats fed varying quantities of soy protein with and without methionine for 28 days - Experiment 3-B

Group	Rat no.	Body wt. g	Liver wt. g	FAO-16 O_2 uptake per min ul	Nitrogen %	Lipid %
S 7.5	535	47	1.77	3.7	2.17	6.87
	544	49	2.06	2.0	1.48	13.51
	523	57	3.05	5.0	2.06	21.47
	579	54	2.37	6.7	2.28	15.10
	587	46	2.48	1.2	1.88	25.85
	599	56	2.50	3.6	2.31	14.26
Mean		52	2.37	3.7	2.03	16.18
S 15.0	542	60	2.42	5.5	2.96	10.15
	569	78	3.22	1.0	2.27	10.61
	582	83	3.37	1.7	2.14	14.52
	520	76	3.65	10.4	2.18	16.23
	594	68	2.30	4.4	2.58	6.60
	598	95	3.48	4.2	2.29	8.64
Mean		77	3.07	4.5	2.40	11.13
S 22.5	533	94	3.78	5.0	2.06	15.97
	561	77	3.36	1.7	2.44	5.24
	570	88	3.34	8.7	2.80	7.38
	517	82	3.08	16.9	2.83	10.30
	528	91	3.91	10.6	2.34	15.60
	577	88	3.40	1.4	2.63	5.40
Mean		87	3.48	7.4	2.52	9.97
S 30.0	563	88	3.50	4.6	2.22	6.63
	584	91	3.28	4.8	3.03	4.79
	522	115	5.32	10.7	2.54	2.63
	527	89	3.60	14.5	2.36	10.14
	586	105	4.64	4.0	2.50	9.88
	593	98	3.83	6.4	2.39	5.18
Mean		98	4.03	7.5	2.51	6.54
SM 7.5	564	70	3.47	7.5	2.43	7.06
	571	65	2.32	4.2	2.40	5.13
	583	61	3.13	3.6	2.48	9.14
	518	85	4.29	11.0	2.10	18.64
	525	62	2.38	7.6	2.53	9.18
	595	61	2.25	1.8	2.30	6.94
Mean		67	2.97	6.0	2.37	9.35
SM 15.0	534	131	4.84	9.4	2.68	7.73
	562	117	4.23	6.5	3.10	5.12
	524	122	5.06	4.0	2.31	8.20

Table 54. (Continued)

Group	Rat no.	Body wt. g	Liver wt. g	FAO-16 O_2 uptake per min ul	Nitrogen %	Lipid %
SM 15.0	526	113	4.44	13.5	2.54	8.37
	578	108	3.75	11.8	3.92	7.59
	588	109	3.81	4.6	2.24	10.42
Mean		117	4.36	8.3	2.80	7.91
SM 22.5	543	118	4.13	13.7	2.56	5.84
	581	105	3.59	10.1	3.49	6.73
	521	141	5.12	10.4	3.07	6.82
	585	142	4.86	8.8	1.76	9.40
	596	144	4.60	5.8	2.59	5.95
	597	136	4.78	3.4	3.10	6.36
Mean		131	4.51	8.7	2.76	6.85
SM 30.0	536	153	6.37	4.9	2.84	4.97
	541	124	4.61	17.4	3.20	4.47
	572	145	5.74	17.4	3.35	5.61
	519	143	5.82	12.4	2.83	5.28
	580	127	4.65	18.0	3.06	5.98
	600	125	4.93	8.6	3.18	5.80
Mean		136	5.35	13.1	3.08	5.35

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